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United States Patent [19]

Edwards

[11] Patent Number: **5,688,936**[45] Date of Patent: **Nov. 18, 1997**[54] **VESICLE MEMBRANE TRANSPORT PROTEINS**[75] Inventor: **Robert H. Edwards**, Los Angeles, Calif.[73] Assignee: **The Regents of the University of California**, Oakland, Calif.[21] Appl. No.: **63,552**[22] Filed: **May 14, 1993****Related U.S. Application Data**

[63] Continuation-in-part of Ser. No. 923,096, Jul. 30, 1992, abandoned, which is a continuation-in-part of Ser. No. 899,074, Jun. 11, 1992, abandoned.

[51] Int. Cl.⁶ **C07H 21/04**; C07K 14/435[52] U.S. Cl. **536/23.5**; 530/350; 530/827

[58] Field of Search 536/23.1, 23.5; 435/320.1, 240.2, 240.4, 252.3, 254.11, 254.2

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Primary Examiner—Stephanie W. Zitomer
Attorney, Agent, or Firm—Merchant, Gould, Smith, Edell, Welter & Schmidt

[57] **ABSTRACT**

Complete cDNA and amino acid sequences are disclosed for rat adrenal-specific and brain-specific transport protein, as well as for human brain-specific transport protein. Methods for obtaining the genes encoding these proteins and for obtaining recombinantly produced protein are described. Antibodies and methods for isolating additional vesicle membrane transport proteins are also described. Methods for using the vesicle membrane transport proteins to identify compounds that selectively inhibit transport of toxic molecules into vesicles, and that prevent inhibition of transport of toxic molecules are also provided. The invention includes methods to treat and diagnose diseases associated with sequestration of toxic molecules in mammalian cells.

1 Claim, 52 Drawing Sheets

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FIG. 1A

1 CCTCGAGATCCATTGTGCTCTAAAGTCAGCACATCCACTTTCAGAGAACAGAGTCTCTGC
61 TGTCTTGCCAACGGCTGCTCCTTCCTCTCTGAGTGCCTCACATCAAGATAAGCTAGAAGT
121 GAGCTTCACTGGACCAGGCAGACTTCTTCTCCTATAAAGGTGACAGAAGACCACATTTGT
181 CGAGGGGTCTTCTAAGCCCTGGGAGGAGAAGCCCCACCATCTCACTCCCTACCCAGCC
241 CAGCCTCCTGCAGCCCTTGCCATGCTCCAGGTTGTTCTGGGTGCTCCTCAGCGGTTGCTG
MetLeuGlnValValLeuGlyAlaProGlnArgLeuLeu
301 AAGGAAGGAAGGCAGTCCCGCAAGCTGGTGCTGGTGGTGGTGTTCGTGGCTCTGCTTCTG
14 LysGluGlyArgGlnSerArgLysLeuValLeuValValValPheValAlaLeuLeuLeu
361 GACAACATGCTGCTCACTGTGGTGGTGCCATTGTGCCACCTTCCTGTACGCGACAGAG
34 AspAsnMetLeuLeuThrValValValProIleValProThrPheLeuTyrAlaThrGlu
421 TTCAAAGACAGCAACTCTTCTCTGCATAGGGGTCCTTCTGTAAGCTCCCAGCAAGCTCTC
54 PheLysAspSerAsnSerSerLeuHisArgGlyProSerValSerSerGlnGlnAlaLeu
*
481 ACCTCTCCTGCCTTCTCTACCATATTCTCCTTCTTTGACAACACCACCAGACTGTAGAA
74 ThrSerProAlaPheSerThrIlePheSerPhePheAspAsnThrThrThrThrValGlu
*
541 GAACATGTACCCTTCCGTGTAAGTGGACAAATGGCACCATCCCTCCTCCAGTCACTGAA
94 GluHisValProPheArgValThrTrpThrAsnGlyThrIleProProProValThrGlu
*
601 GCCAGCTCAGTACCAAAAAACAAGTCTTGCAAGGGATAGAGTTCTTAGAAGAAGAAAAC
114 AlaSerSerValProLysAsnAsnCysLeuGlnGlyIleGluPheLeuGluGluGluAsn
661 GTTCGGATTGGGATTCTATTTGCTTCAAAGCTTTGATGCAACTTCTGGTCAACCCATTT
134 ValArgIleGlyIleLeuPheAlaSerLysAlaLeuMetGlnLeuLeuValAsnProPhe
721 GTAGGACCTCTTACTAACAGGATTGGCTATCACATCCCATGTTTGTGGCTTTATGATC
154 ValGlyProLeuThrAsnArgIleGlyTyrHisIleProMetPheValGlyPheMetIle
781 ATGTTTCTCTCCACACTAATGTTTGTCTTCTCTGGCACCTATGCCCTGCTATTTGTGGCC
174 MetPheLeuSerThrLeuMetPheAlaPheSerGlyThrTyrAlaLeuLeuPheValAla
841 CGAACTCTCCAAGGCATTGGATCTTCGTTTTTCATCTGTTGCAGGACTTGGGATGCTGGCC
194 ArgThrLeuGlnGlyIleGlySerSerPheSerSerValAlaGlyLeuGlyMetLeuAla

FIG. 1B

901 AGTGTCTATACTGACAACTATGAGAGAGGGAGAGCCATGGGAATTGCTTTGGGGGGCCTG
214 SerValTyrThrAspAsnTyrGluArgGlyArgAlaMetGlyIleAlaLeuGlyGlyLeu

961 GCCTTGGGACTTCTGGTGGGAGCACCTTTCGGAAGTGTGATGTATGAATTTGTGGGCAAG
234 AlaLeuGlyLeuLeuValGlyAlaProPheGlySerValMetTyrGluPheValGlyLys

1021 TCCTCACCATTCTCATCTTGGCCTTCTTGGCACTTCTGGATGGAGCTCTCCAACCTTGC
254 SerSerProPheLeuIleLeuAlaPheLeuAlaLeuLeuAspGlyAlaLeuGlnLeuCys

1081 ATCCTATGGCCTTCGAAAGTGTCTCCTGAGAGTGCCATGGGGACTTCGCTTTTGACGCTT
274 IleLeuTrpProSerLysValSerProGluSerAlaMetGlyThrSerLeuLeuThrLeu

1141 CTCAAAGACCCTTACATCCTGGTAGCAGCAGGTTCCATCTGCTTGGCCAACATGGGAGTC
294 LeuLysAspProTyrIleLeuValAlaAlaGlySerIleCysLeuAlaAsnMetGlyVal

1201 GCCATACTAGAGCCCACGCTGCCCATCTGGATGATGCAGACCATGTGCTCCCCGAGTGG
314 AlaIleLeuGluProThrLeuProIleTrpMetMetGlnThrMetCysSerProGluTrp

1261 CAGCTAGGTCTGGCTTTCTTGCCTGCTAGTGTGGCCTACCTCATTGGCACGAACCTCTTT
334 GlnLeuGlyLeuAlaPheLeuProAlaSerValAlaTyrLeuIleGlyThrAsnLeuPhe

1321 GGTGTGTTGGCTAACAAGATGGGTTCGGTGGCTGTGCTCCCTTGTTGGGATGGTGGCAGTA
354 GlyValLeuAlaAsnLysMetGlyArgTrpLeuCysSerLeuValGlyMetValAlaVal

1381 GGTATCAGCTTGCTCTGTGTACCTCTGGCTCACAATATTTTTGGTCTTATTGGCCCCAAT
374 GlyIleSerLeuLeuCysValProLeuAlaHisAsnIlePheGlyLeuIleGlyProAsn

1441 GCAGGCCTTGGCTTTGCCATAGGAATGGTGGATTCCTCTCTGATGCCATCATGGGATAC
394 AlaGlyLeuGlyPheAlaIleGlyMetValAspSerSerLeuMetProIleMetGlyTyr

1501 CTGGTGGACTTACGCCACACCTCTGTGTATGGGAGTGTCTATGCCATCGCCGATGTGGCC
414 LeuValAspLeuArgHisThrSerValTyrGlySerValTyrAlaIleAlaAspValAla

1561 TTTTGTGTGGGCTTTGCTATTGGCCCATCTACTGGGGGTGTTATCGTACAGGTCATTGGC
434 PheCysValGlyPheAlaIleGlyProSerThrGlyGlyValIleValGlnValIleGly

1621 TTTCCTTGGCTCATGGTCATCATTGGTACCATCAACATCATTATGCTCCTCTCTGCTGC
454 PheProTrpLeuMetValIleIleGlyThrIleAsnIleIleTyrAlaProLeuCysCys

FIG. 1C

1681 TTCCTGCAGAACCCGCCAGCTAAGGAGGAGAAGCGTGCAATTCTGAGCCAGGAATGCCCC

474 PheLeuGlnAsnProProAlaLysGluGluLysArgAlaIleLeuSerGlnGluCysPro

1741 ACAGAGACCCAGATGTACACATTCCAGAAGCCCACAAAGGCGTTTCCACTAGGAGAGAAC

494 ThrGluThrGlnMetTyrThrPheGlnLysProThrLysAlaPheProLeuGlyGluAsn

1801 AGCGATGATCCTAGCAGCGGGGAGTAACTGCGGAGGGCGATATCTGAGCCTCACATCTAC

514 SerAspAspProSerSerGlyGlu [SEQ ID NO : 2]

1861 AGGGACCAGTCTACTACAGATTCAATAATTTTCACTTTCCTCTCCTCCAGGCCACTGCCT

1921 TCCTCCCTTCTTATTGATACCTTTCCTTTACTCACCTGTAAGTGCAACCCACCACTCTCC

1981 CTCTGTGCTTTGACACCACCCATGGCCCACTTTTTGTGGGAGGACAGTGCTATTTCTGC

2041 CAGGCCAAAGCGAAGCTGATTAAAGCTGAGTTGTGACAAGTTCTGCAAGGGGTGACTCAC

2101 TTCCTGCAGGCAGGACTGAACAATGTGCCTGCGAAATCAGGGGGACAAATGACAAGCCTG

2161 CCTTTCTTCTCTGATTGTTTTTTTTTTTTTTTTTGACATATTACCAATATGTCCTAAAATT

2221 TGACTIONGTCCTGTGAAATGCTTCCCCTTATTTTTTCCAGTTTAGCTTCTATAACATAC

2281 GGGTTTTTGCTTATTTTATGTGCTAAAATTGTTTACCTTCATTAAGTGAGGCCTTCCTAC

2341 TTTCTTCATCGCCCAATTGAGAGGAAATAAACAACTTTCTTAGGCTTGAAAAAAAACCTTT

2401 AGAGCACAATGGATCTCGAGG [SEQ ID NO : 1]

FIG. 2A

1 GGGCGCACGGACAGAGACCCAGGCTGTGTGGCGCTATAACCGCGCAGTCACAGGCGAGC

60 CAGAGCAGAGCCATGGCCCTGAGCGATCTGGTGCTGCTGCGATGGCTGCCGGACAGCCGC
[SEQ ID NO : 4] MetAlaLeuSerAspLeuValLeuLeuArgTrpLeuArgAspSerArg

119 CACTCGCGCAAACCTGATCCTGTTCATCGTGTTCCTTGCGCTGCTGCTGGACAACATGCTG
17 HisSerArgLysLeuIleLeuPheIleValPheLeuAlaLeuLeuLeuAspAsnMetLeu

179 CTCACCGTCGTGGTTCATCATCCCCAGCTATCTGTACAGCATTAAAGCATGAGAAAAAC
37 LeuThrValValValProIleIleProSerTyrLeuTyrSerIleLysHisGluLysAsn
*

239 TCTACGGAAATCCAGACCACCAGACCAGAGCTCGTGGTCTCCACCTCCGAAAGCATCTTC
57 SerThrGluIleGlnThrThrArgProGluLeuValValSerThrSerGluSerIlePhe

299 TCTTACTATAACAACCTCTACTGTGTTGATCACCGGGAATGCCACTGGGACTCTTCCAGGA
77 SerTyrTyrAsnAsnSerThrValLeuIleThrGlyAsnAlaThrGlyThrLeuProGly
* *

359 GGGCAGTCACACAAGGCTACCAGCACACAGCACACTGTGGCTAACACCACTGTCCCTTCG
97 GlyGlnSerHisLysAlaThrSerThrGlnHisThrValAlaAsnThrThrValProSer
*

419 GACTGTCCCAGTGAAGACAGAGACCTTCTGAATGAGAATGTGCAAGTTGGGCTGCTGTTT
117 AspCysProSerGluAspArgAspLeuLeuAsnGluAsnValGlnValGlyLeuLeuPhe

479 GCCTCAAAGCCACTGTCCAGCTCCTCACTAACCATTTCATAGGACTTCTGACCAACAGA
137 AlaSerLysAlaThrValGlnLeuLeuThrAsnProPheIleGlyLeuLeuThrAsnArg

539 ATTGGCTATCCAATTCCCATGTTTGCCGGCTTCTGCATCATGTTTATCTCAACAGTTATG
157 IleGlyTyrProIleProMetPheAlaGlyPheCysIleMetPheIleSerThrValMet

599 TTTGCCTTCTCCAGCAGCTATGCCTTCTGCTGATCGCCAGGTCCCTTCAGGGAATTGGC
177 PheAlaPheSerSerSerTyrAlaPheLeuLeuIleAlaArgSerLeuGlnGlyIleGly

659 TCCTCCTGCTCATCCGTGGCTGGGATGGGTATGCTGGCCAGCGTGACACAGATGATGAG
197 SerSerCysSerSerValAlaGlyMetGlyMetLeuAlaSerValTyrThrAspAspGlu

719 GAGAGGGGGAAGCCCATGGGCATTGCTTTGGGTGGCCTGGCCATGGGAGTCTTAGTGGGA
217 GluArgGlyLysProMetGlyIleAlaLeuGlyGlyLeuAlaMetGlyValLeuValGly

779 CCCCCCTTCGGGAGTGTGCTCTATGAGTTTGTGGGGAAGACAGCTCCCTTCTGGTGCTA
237 ProProPheGlySerValLeuTyrGluPheValGlyLysThrAlaProPheLeuValLeu

FIG. 2 B

839 GCTGCCTTGGTGCTCTTGGATGGGGCTATTCAGCTCTTTGTGCTCCAGCCGTC^{CCGAGTA}
257 AlaAlaLeuValLeuLeuAspGlyAlaIleGlnLeuPheValLeuGlnProSerArgVal

899 CAGCCAGAGAGTCAGAAGGGGACACCTCTAACGACCTTGCTGAAGGATCCATACATCCTC
277 GlnProGluSerGlnLysGlyThrProLeuThrThrLeuLeuLysAspProTyrIleLeu

959 ATCGCTGCAGGCTCCATCTGCTTTGCAAACATGGGGATAGCCATGCTGGAGCCCGCCCTG
297 IleAlaAlaGlySerIleCysPheAlaAsnMetGlyIleAlaMetLeuGlyProAlaLeu

1019 CCCATCTGGATGATGGAGACCATGTGTTCCCGAAAGTGGCAGCTGGGCGTTGCTTTCCTC
317 ProIleTrpMetMetGluThrMetCysSerArgLysTrpGlnLeuGlyValAlaPheLeu

1079 CCGGCGAGCATCTCTTATCTCATTGGAACCAATATTTTTGGGATACTTGCACACAAAATG
337 ProAlaSerIleSerTyrLeuIleGlyThrAsnIlePheGlyIleLeuAlaHisLysMet

1139 GGAAGGTGGCTATGTGCTCTTCTGGGAATGGTAATTGTTGGAATCAGCATT^{TTTATGCATC}
357 GlyArgTrpLeuCysAlaLeuLeuGlyMetValIleValGlyIleSerIleLeuCysIle

1199 CCCTTTGCAAAAATATCTATGGACTCATCGCTCCCACTTTGGAGTTGGTTTTGCAATT
377 ProPheAlaLysAsnIleTyrGlyLeuIleAlaProAsnPheGlyValGlyPheAlaIle

1259 GGGATGGTGGACTCCTCTATGATGCCTATCATGGGCTACCTGGTTGACCTGCGGCATGTG
397 GlyMetValAspSerSerMetMetProIleMetGlyTyrLeuValAspLeuArgHisVal

1319 TCTGTCTATGGGAGTGTTTATGCCATTGCAGACGTGGCCTTTTGTATGGGCTATGCTATC
417 SerValTyrGlySerValTyrAlaIleAlaAspValAlaPheCysMetGlyTyrAlaIle

1379 GGTCCCTCTGCTGGTGGTGCCATCGCAAAGGCAATTGGCTTTCCTTGGCTTATGACAATT
437 GlyProSerAlaGlyGlyAlaIleAlaLysAlaIleGlyPheProTrpLeuMetThrIle

1439 ATTGGGATAATTGATATCGCTTTTGCTCCACTCTGCTTTTTCCTTCGAAGTCCACCTGCT
457 IleGlyIleIleAspIleAlaPheAlaProLeuCysPhePheLeuArgSerProProAla

1499 AAGGAGGAAAAAATGGCTATCCTCATGGACCACAAC^{TGTCCCATTAAAGAAAGATGTAC}
477 LysGluGluLysMetAlaIleLeuMetAspHisAsnCysProIleLysThrLysMetTyr

1559 ACTCAGAATAATGTCCAGTCATATCCCATCGGTGATGATGAAGAATCTGAAAGTGA^{CTGA}
497 ThrGlnAsnAnsValGlnSerTyrProIleGlyAspAspGluGluSerGluSerAsp***

1619 GACCCTCTAACGTCGCC [SEQ ID NO : 3]

FIG. 3 A

1 GGCGCAAGCGACCCCGAGCGGAGCCCGGAGCCATGGCCCTGAGCGAGCTGGCGCTGGTC
MetAlaLeuSerGluLeuAlaLeuVal

61 CGCTGGCTGCAGGAGAGCCGCCGCTCGCGGAAGCTCATCCTGTTTCATCGTGTTCTGGCG
10 ArgTrpLeuGlnGluSerArgArgSerArgLysLeuIleLeuPheIleValPheLeuAla

121 CTGCTGCTGGACAACATGCTGCTCACTGTCTGGTCCCCATCATCCAAGTTATCTGTAC
30 LeuLeuLeuAspAsnMetLeuLeuThrValValValProIleIleProSerTyrLeuTyr

181 AGCATTAAGCATGAGAAGAATGCTACAGAAATCCAGACGGCCAGGCCAGTGCACACTGCC
50 SerIleLysHisGluLysAsnAlaThrGluIleGlnThrAlaArgProValHisThrAla
*

241 TCCATCTCAGACAGCTTCCAGAGCATCTTCTCCTATTATGATAACTCGACTATGGTCACC
70 SerIleSerAspSerPheGlnSerIlePheSerTyrTyrAspAsnSerThrMetValThr
*

301 GGGAATGCTACCAGAGACCTGACACTTCATCAGACCGCCACACAGCACATGGTGACCAAC
90 GlyAsnAlaThrArgAspLeuThrLeuHisGlnThrAlaThrGlnHisMetValThrAsn
*

361 GCGTCCGCTGTTCTTCCGACTGTCCAGTGAAGACAAAGACCTCCTGAATGAAAACGTG
110 AlaSerAlaValProSerAspCysProSerGluAspLysAspLeuLeuAsnGluAsnVal
*

421 CAAGTTGGTCTGTTGTTTGCCTCGAAAGCCACCGTCCAGCTCATCACCAACCCTTTCATA
130 GlnValGlyLeuLeuPheAlaSerLysAlaThrValGlnLeuIleThrAsnProPheIle

481 GGACTACTGACCAACAGAATTGGCTATCCAATCCCATATTTGCGGGATTCTGCATCATG
150 GlyLeuLeuThrAsnArgIleGlyTyrProIleProIlePheAlaGlyPheCysIleMet

541 TTTGTCTCAACAATTATGTTTGCCTTCTCCAGCAGCTATGCCTTCTGCTGATTGCCAGG
170 PheValSerThrIleMetPheAlaPheSerSerSerTyrAlaPheLeuLeuIleAlaArg

601 TCGCTGCAGGGCATCGGCTCGTCCTGCTCCTCTGTGGCTGGGATGGGCATGCTTGCCAGT
190 SerLeuGlnGlyIleGlySerSerCysSerSerValAlaGlyMetGlyMetLeuAlaSer

661 GTCTACACAGATGATGAAGAGAGAGGCAACGTCATGGGAATCGCCTTGGGAGGCCTGGCC
210 ValTyrThrAspAspGluGluArgGlyAsnValMetGlyIleAlaLeuGlyGlyLeuAla

721 ATGGGGGTCTTAGTGGGCCCCCCTTCGGGAGTGTGCTCTATGAGTTTGTGGGGAAGACG
230 MetGlyValLeuValGlyProProPheGlySerValLeuTyrGluPheValGlyLysThr

FIG. 3 B

781 GCTCCGTTCTGGTGCTGGCCGCCCTGGTACTCTTGGATGGAGCTATTCAGCTCTTTGTG
250 AlaProPheLeuValLeuAlaAlaLeuValLeuLeuAspGlyAlaIleGlnLeuPheVal

841 CTCCAGCCGTCCCGGGTGCAGCCAGAGAGTCAGAAGGGGACACCCCTAACCACGCTGCTG
270 LeuGlnProSerArgValGlnProGluSerGlnLysGlyThrProLeuThrThrLeuLeu

901 AAGGACCCGTACATCCTCATTGCTGCAGGCTCCATCTCCTTTGCAAACATGGGCATCGCC
290 LysAspProTyrIleLeuIleAlaAlaGlySerIleCysPheAlaAsnMetGlyIleAla

961 ATGCTGGAGCCAGCCCTGCCCATCTGGATGATGGAGACCATGTGTTCCTCCGCAAGTGGCAG
310 MetLeuGluProAlaLeuProIleTrpMetMetGluThrMetCysSerArgLysTrpGln

1021 CTGGGCGTTGCCTTCTTGCCAGCTAGTATCTCTTATCTCATTGGAACCAATATTTTTGGG
330 LeuGlyValAlaPheLeuProAlaSerIleSerTyrLeuIleGlyThrAsnIlePheGly

1081 ATACTTGCACACACAATGGGGAGGTGGCTTTGTGCTCTTCTGGGAATGATAATTGTTGGA
350 IleLeuAlaHisLysMetGlyArgTrpLeuCysAlaLeuLeuGlyMetIleIleValGly

1141 GTCAGCATTTTATGTATTCCATTTCCAAAAACATTTATGGACTCATAGCTCCGAACTTT
370 ValSerIleLeuCysIleProPheAlaLysAsnIleTyrGlyLeuIleAlaProAsnPhe

1201 GGAGTTGGTTTTGCAAATGGAATGGTGGATTCGTCAATGATGCCTATCATGGGCTACCTC
390 GlyValGlyPheAlaIleGlyMetValAspSerSerMetMetProIleMetGlyTyrLeu

1261 GTAGACCTGCGGCACGTGTCCGTCTATGGGAGTGTGTACGCCATTGCGGATGTGGCATT
410 ValAspLeuArgHisValSerValTyrGlySerValTyrAlaIleAlaAspValAlaPhe

1321 TGTATGGGGTATGCTATAGGTCCTTCTGCTGGTGGTCTATTGCAAAGGCAATTGGATTT
430 CysMetGlyTyrAlaIleGlyProSerAlaGlyGlyAlaIleAlaLysAlaIleGlyPhe

1381 CCATGGCTCATGACAATTATTGGGATAATTGATATTCTTTTTGCCCTCTCTGCTTTTTT
450 ProTrpLeuMetThrIleIleGlyIleIleAspIleLeuPheAlaProLeuCysPhePhe

1441 CTTCGAAGTCCACCTGCCAAAGAAGAAAAATGGCTATTCTCATGGATCACAACCTGCCCT
470 LeuArgSerProProAlaLysGluGluLysMetAlaIleLeuMetAspHisAsnCysPro

1501 ATTAAAACAAAAATGTACACTCAGAATAATATCCAGTCATATCCGATAGGTGAAGATGAA
490 IleLysThrLysMetTyrThrGlnAsnAsnIleGlnSerTyrProIleGlyGluAspGlu

FIG. 3 C

1561 GAATCTGAAAGTGACTGAGATGAGATCCTCAAAAATCATCAAAGTGTTTAATTGTATAAA
510 GluSerGluSerAsp***

1621 ACAGTGTTTCCAGTGACACAACCTCATCCAGAAGTGTCTTAGTCATACCATCCATCCCTGG

1681 TGAAAGAGTAAAACCAAAGGTTATTATTTCTTTCCATGGTTATGGTCGATTGCCAACAG

1141 GTCAGCATTTTATGTATTCCATTTCCAAAAACATTTATGGACTCATAGCTCCGAACTTT

370 ValSerIleLeuCysIleProPheAlaLysAsnIleTyrGlyLeuIleAlaProAsnPhe

1201 GGAGTTGGTTTTGCAAATGGAATGGTGGATTTCGTCAATGATGCCTATCATGGGCTACCTC

390 GlyValGlyPheAlaIleGlyMetValAspSerSerMetMetProIleMetGlyTyrLeu

1261 GTAGACCTGCGGCACGTGTCCGTCTATGGGAGTGTGTACGCCATTGCGGATGTGGCATT

410 ValAspLeuArgHisValSerValTyrGlySerValTyrAlaIleAlaAspValAlaPhe

1321 TGTATGGGGTATGCTATAGGTCCTTCTGCTGGTGGTGCTATTGCAAAGGCAATTGGATTT

430 CysMetGlyTyrAlaIleGlyProSerAlaGlyGlyAlaIleAlaLysAlaIleGlyPhe

1381 CCATGGCTCATGACAATTATTGGGATAATTGATATTCTTTTTGCCCTCTCTGCTTTTTTT

450 ProTrpLeuMetThrIleIleGlyIleIleAspIleLeuPheAlaProLeuCysPhePhe

1441 CTTCGAAGTCCACCTGCCAAAGAAGAAAAAATGGCTATTCTCATGGATCACAACCTGCCCT

470 LeuArgSerProProAlaLysGluGluLysMetAlaIleLeuMetAspHisAsnCysPro

1501 ATTAAAACAAAAATGTACACTCAGAATAATATCCAGTCATATCCGATAGGTGAAGATGAA

490 IleLysThrLysMetTyrThrGlnAsnAsnIleGlnSerTyrProIleGlyGluAspGlu

1561 GAATCTGAAAGTGACTGAGATGAGATCCTCAAAAATCATCAAAGTGTTTAATTGTATAAA

510 GluSerGluSerAsp*** [SEQ ID NO : 13]

1621 ACAGTGTTTCCAGTGACACAACCTCATCCAGAAGTGTCTTAGTCATACCATCCATCCCTGG

1681 TGAAAGAGTAAAACCAAAGGTTATTATTTCTTTCCATGGTTATGGTCGATTGCCAACAG

1741 CCTTATAAAGAAAAAGAAGCTTTTCTAGGGGTTTGTATAAATAGTGTTGAAACTTTATTT

1801 TATGTATTTAATTTTATTAAATATCATACAATATATTTTGATGAAATAGGTATTGTGTAA

1861 ATCTATAAATATTTGAATCCAAACCAAATATAATTTCC [SEQ ID NO : 12]

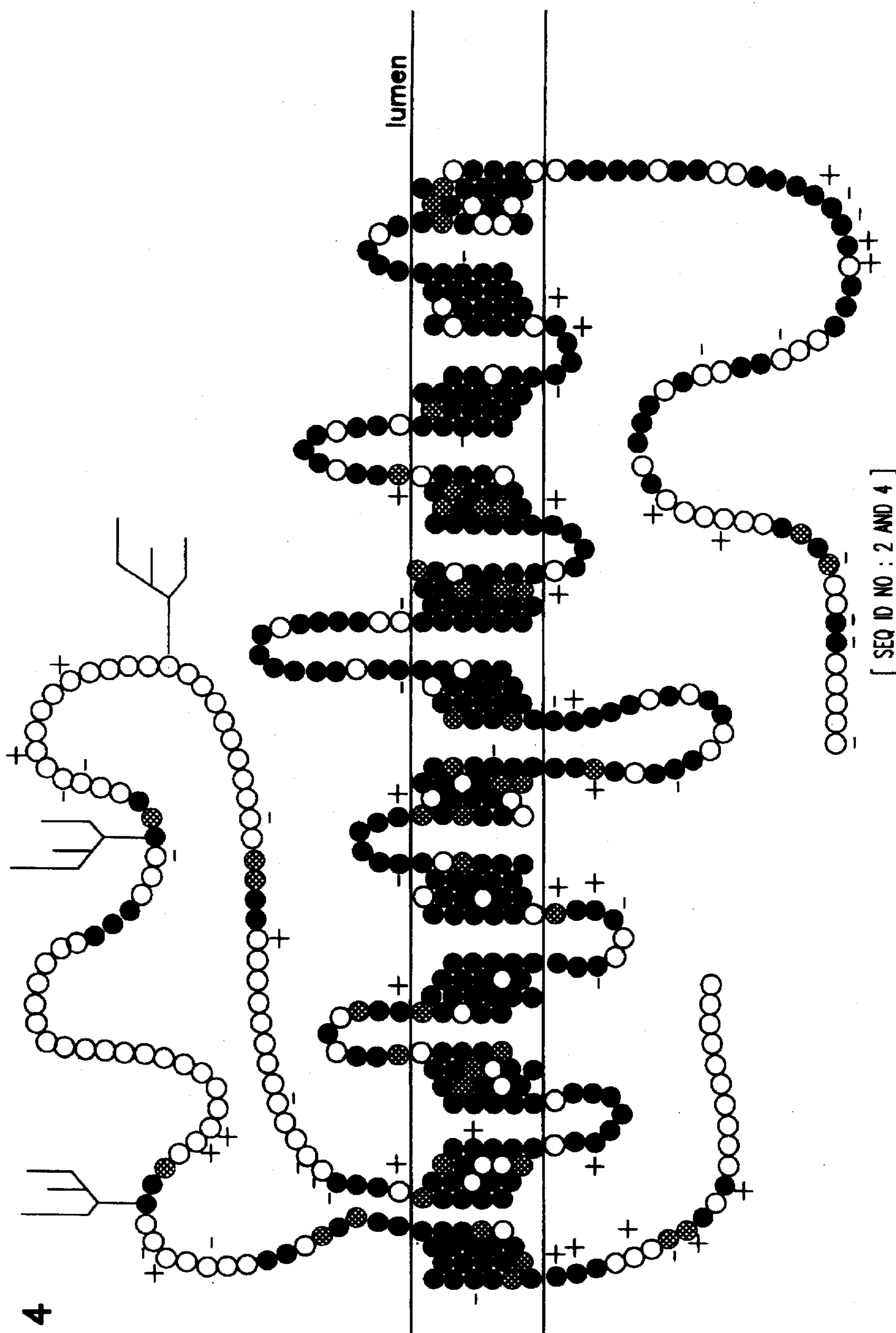


FIG. 4

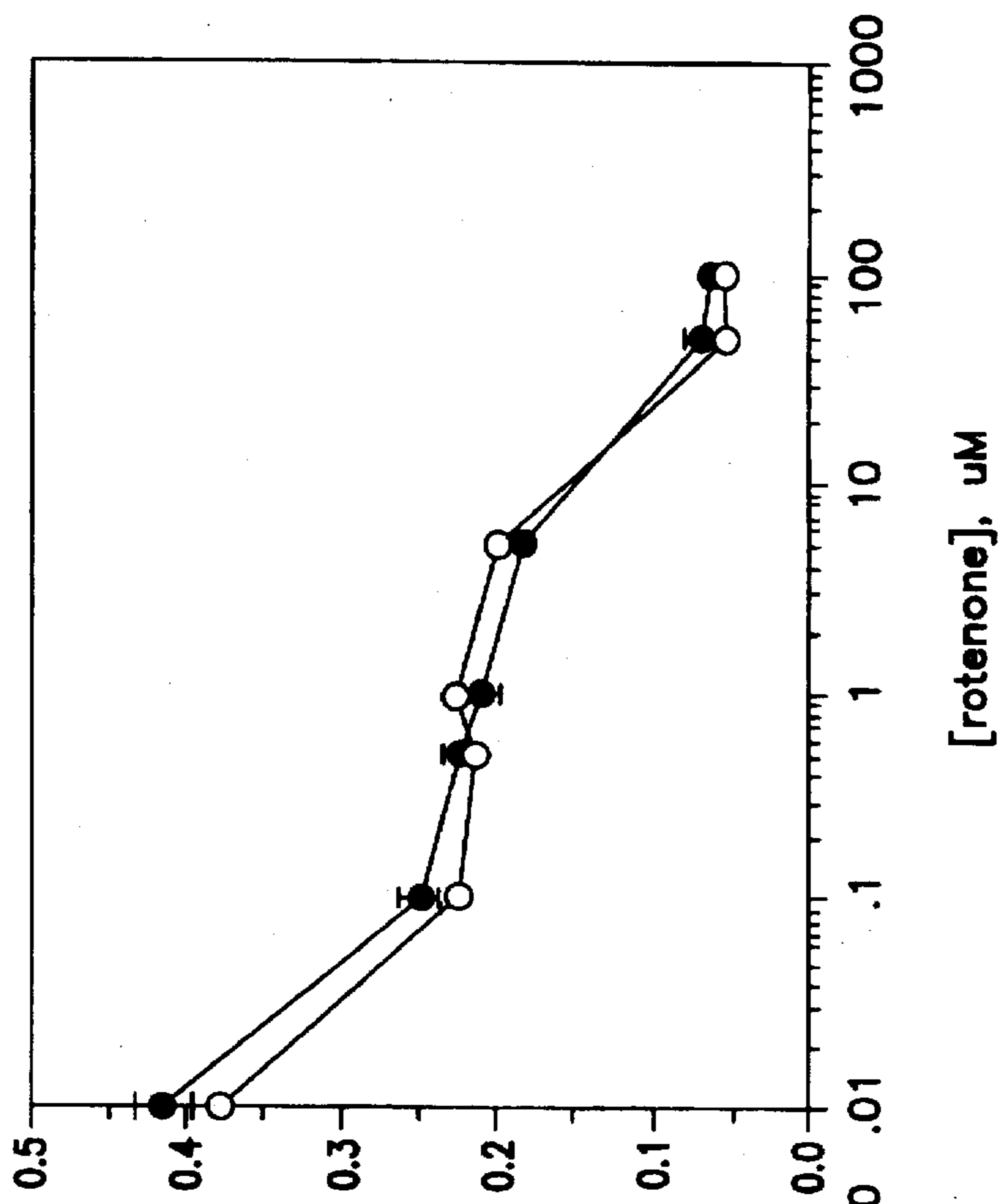


FIG. 6B

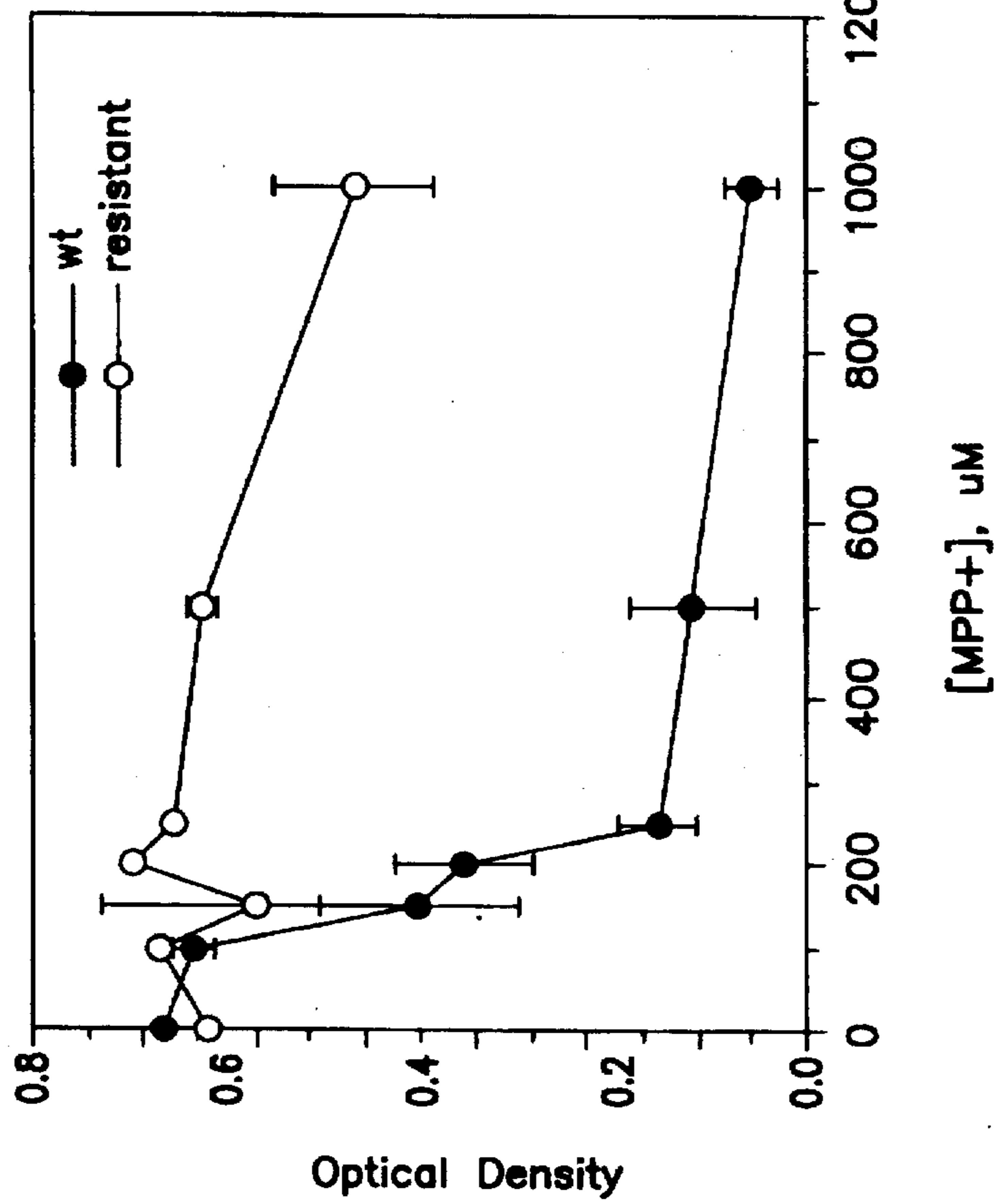


FIG. 6A

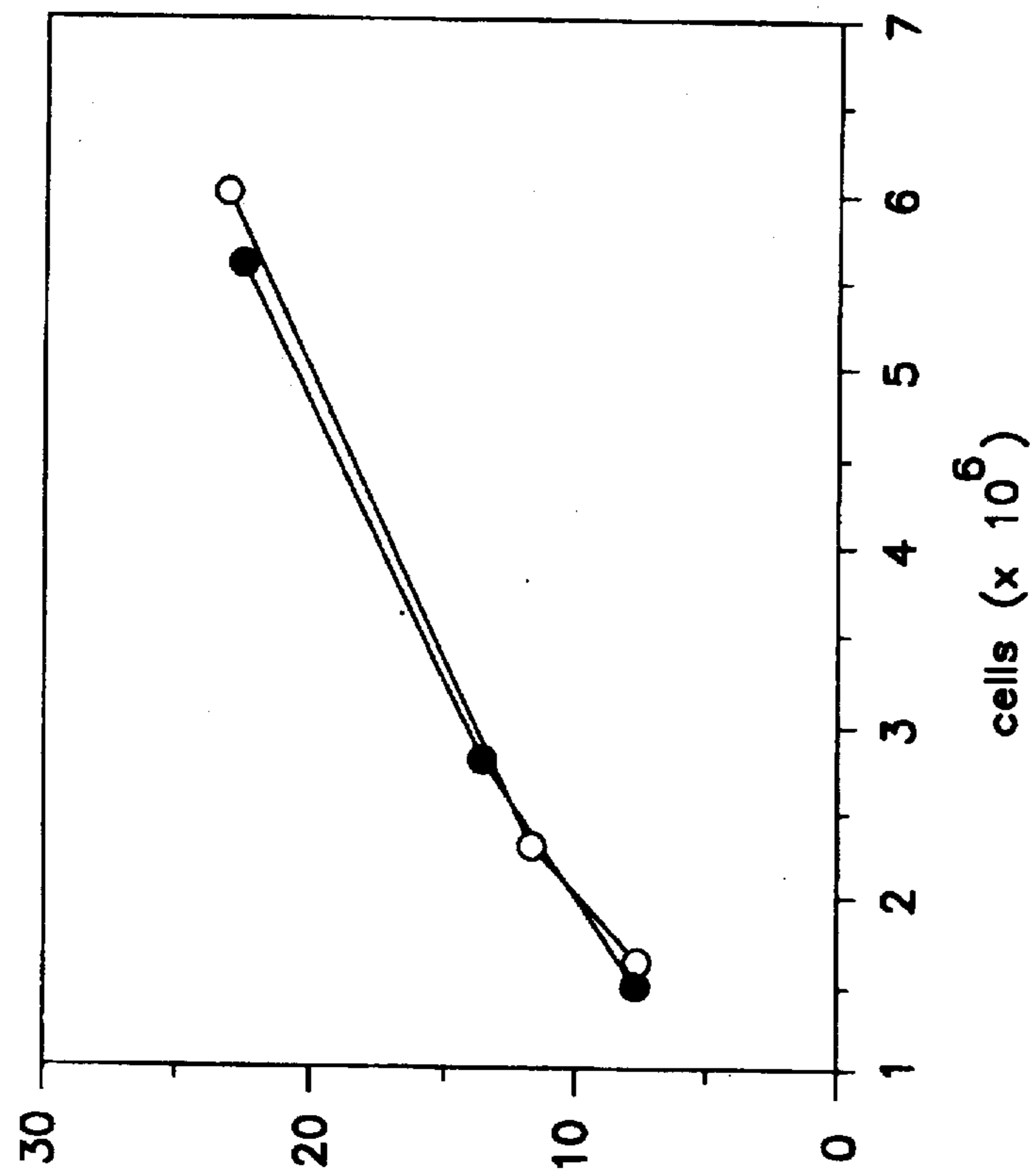


FIG. 7B

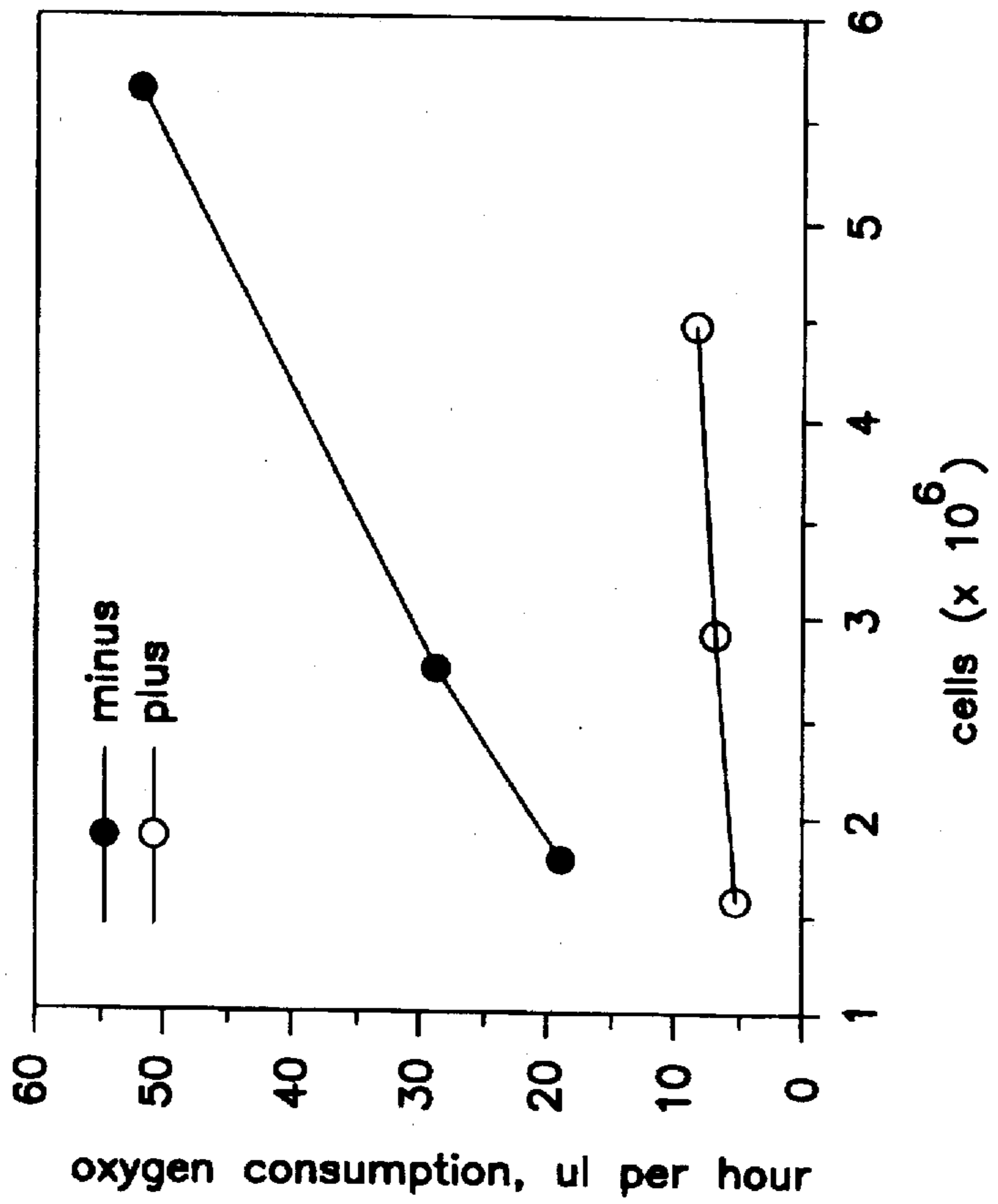


FIG. 7A

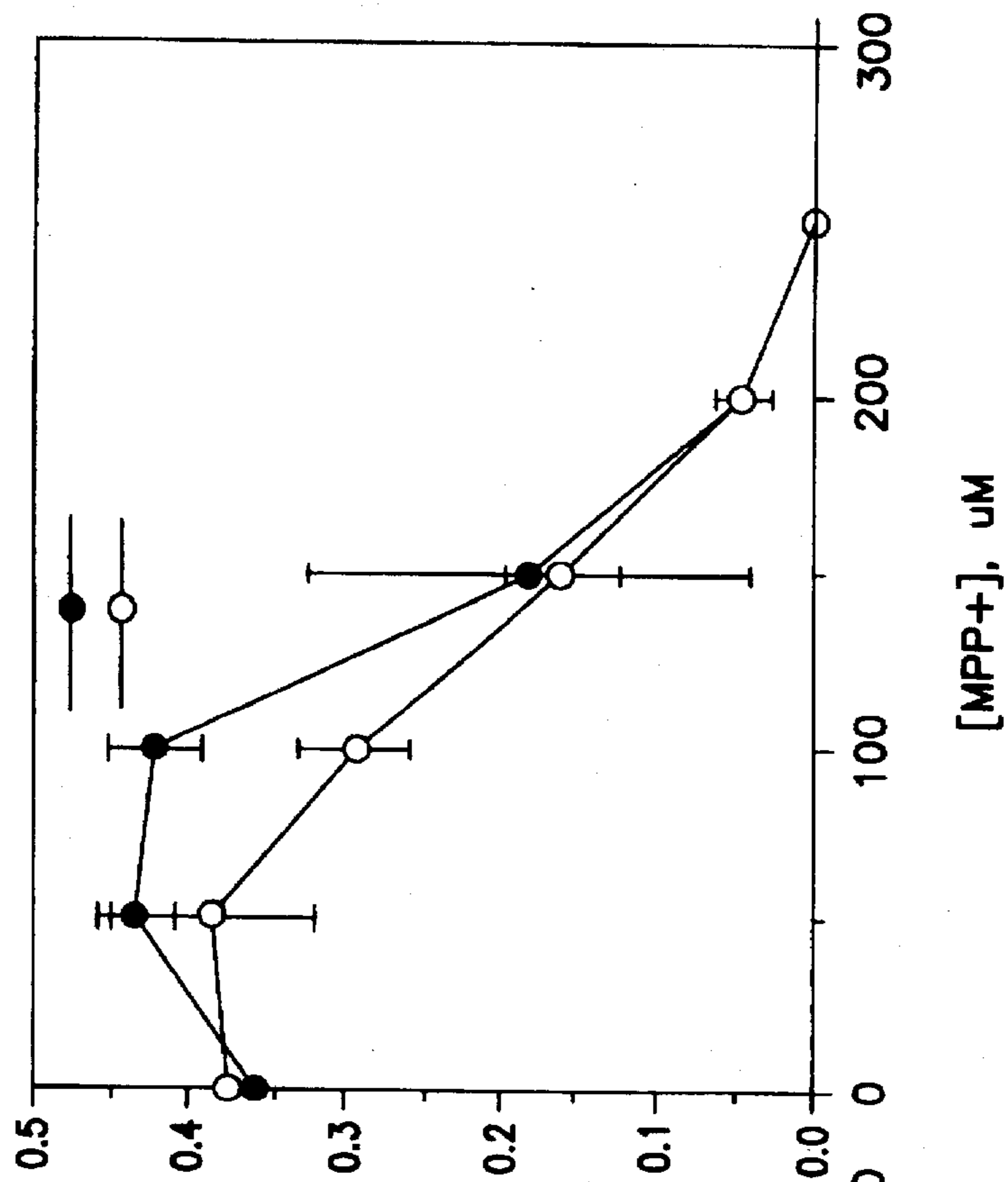


FIG. 8B

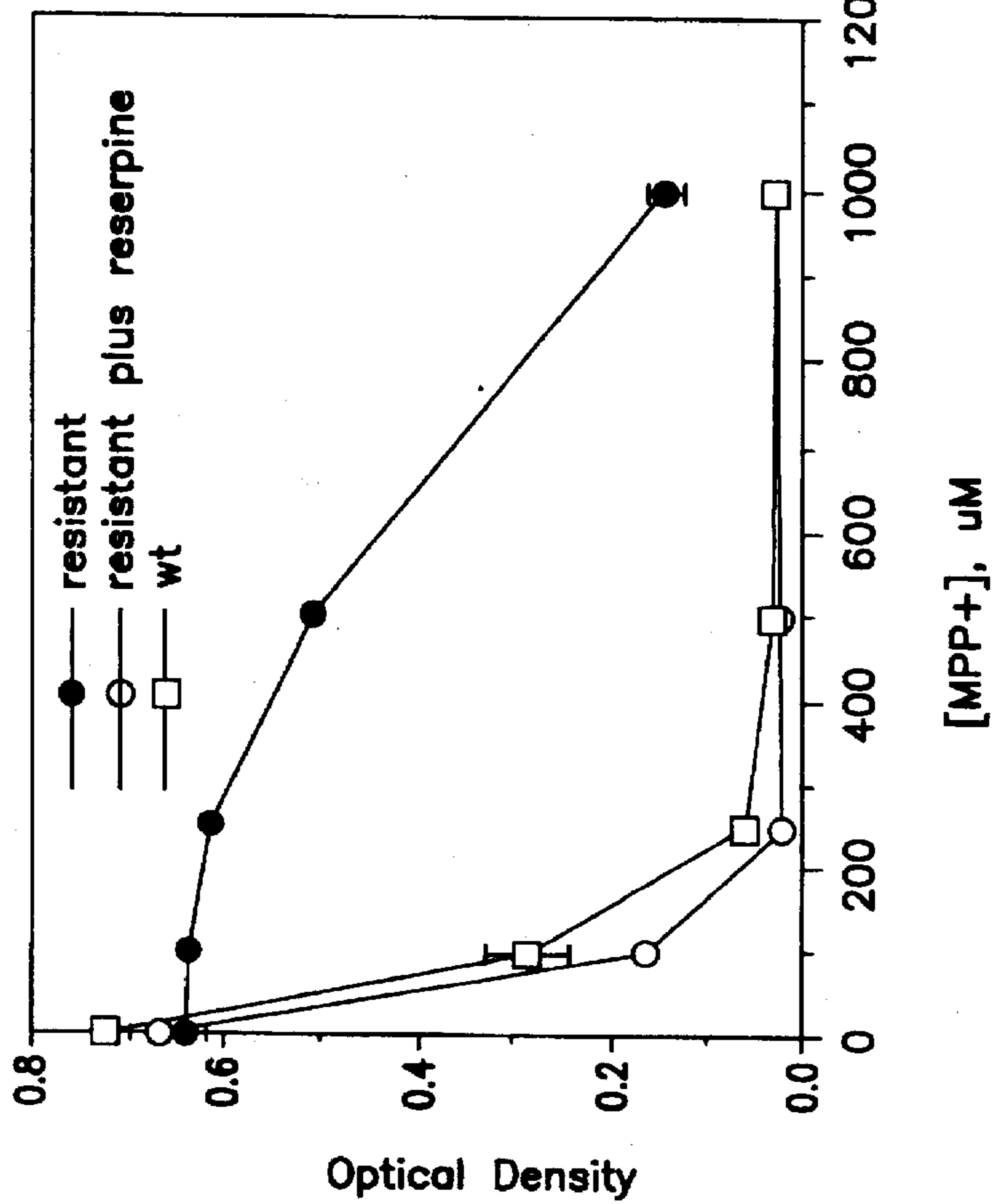


FIG. 8A

FIG. 9A

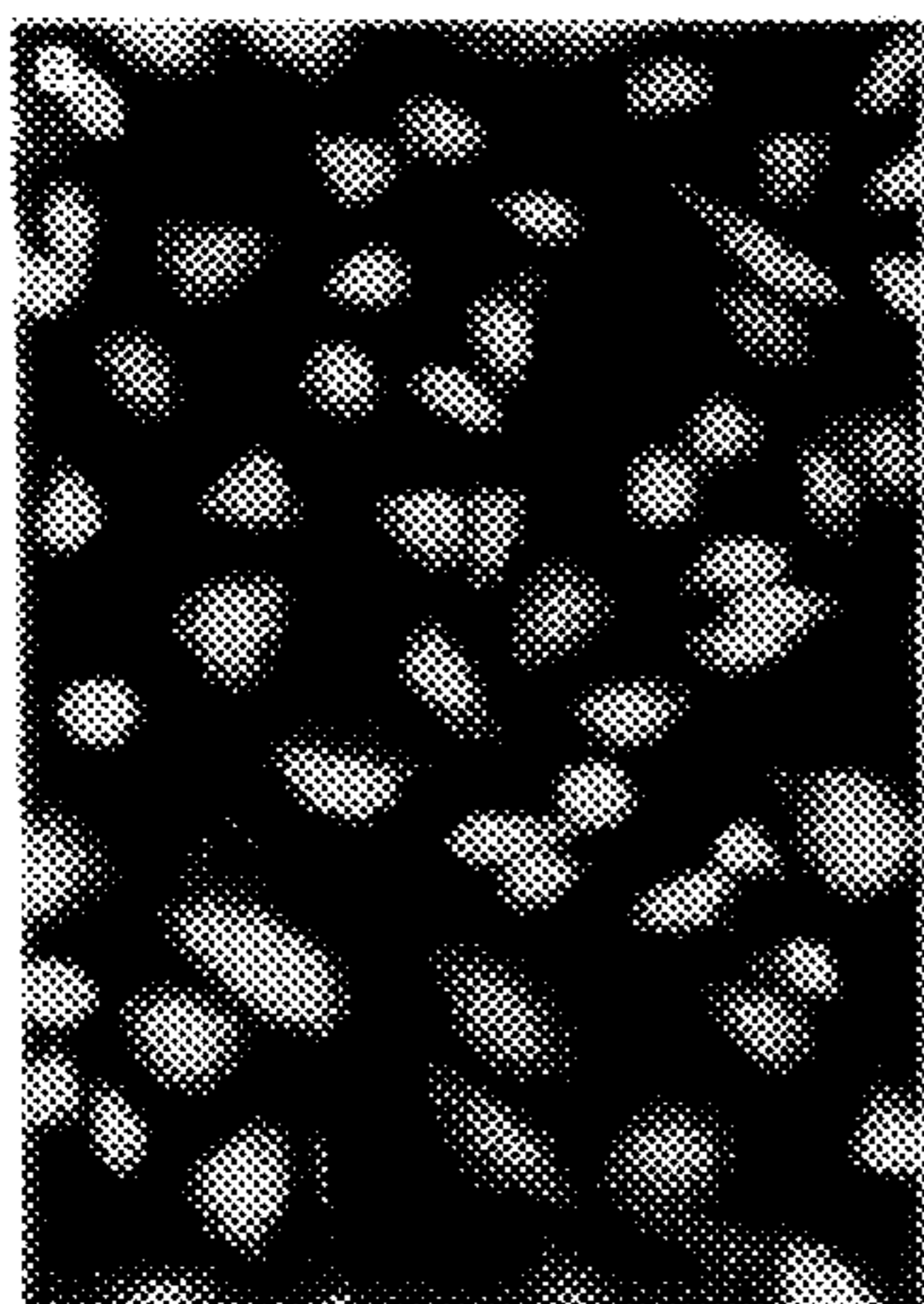


FIG. 9B



FIG. 9C



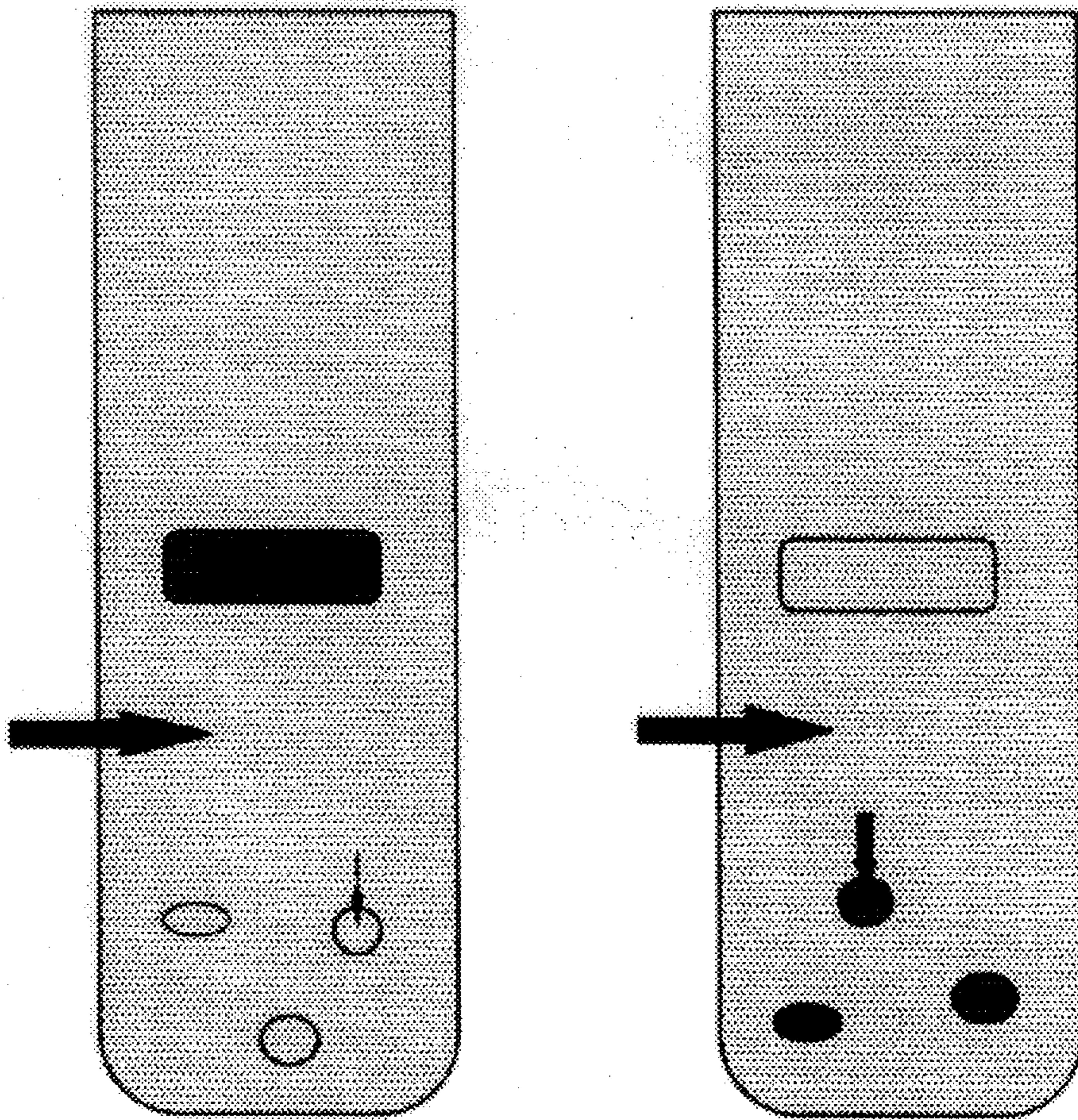


FIG. 10

FIG. II

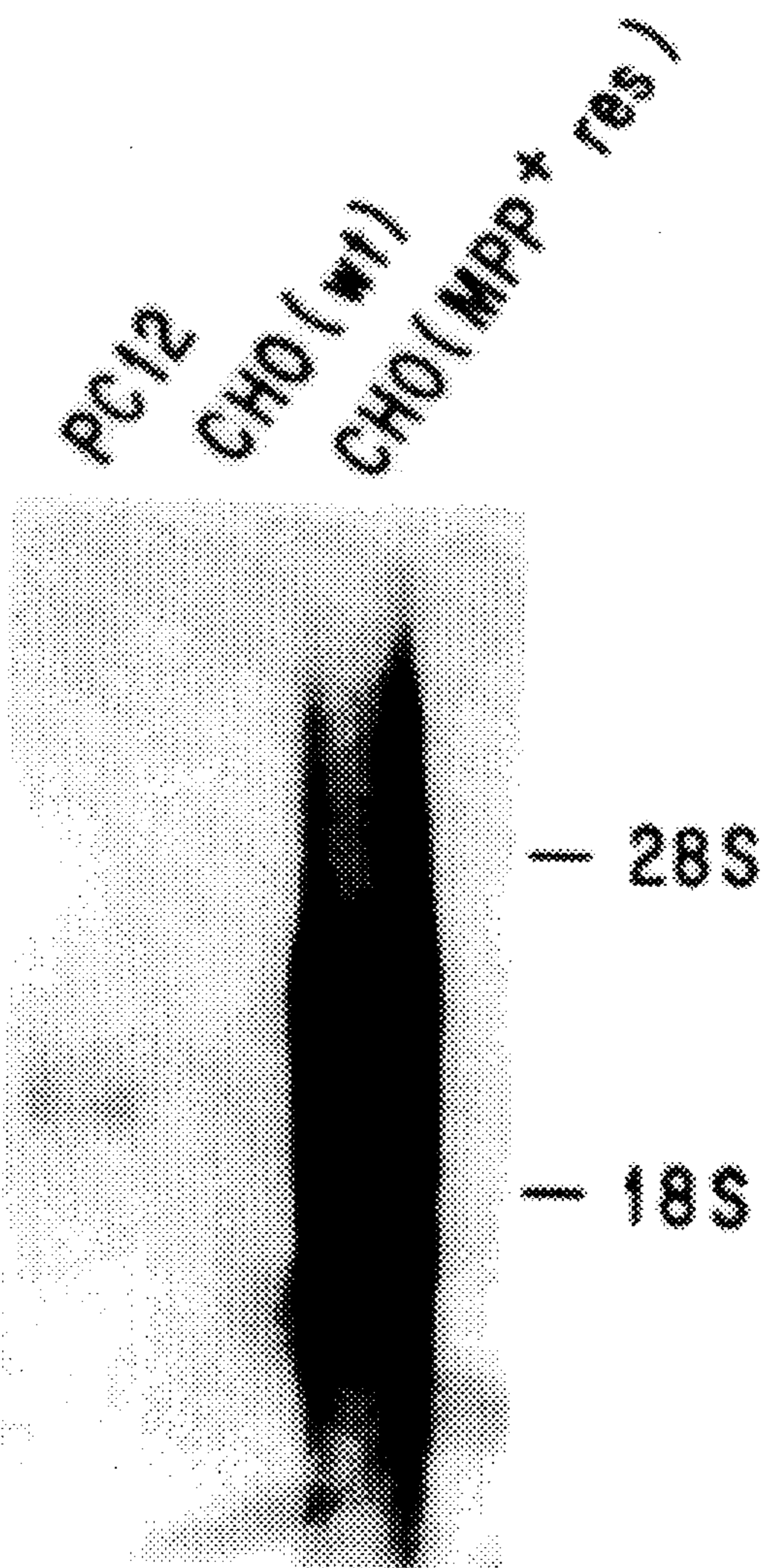


FIG. 12 A

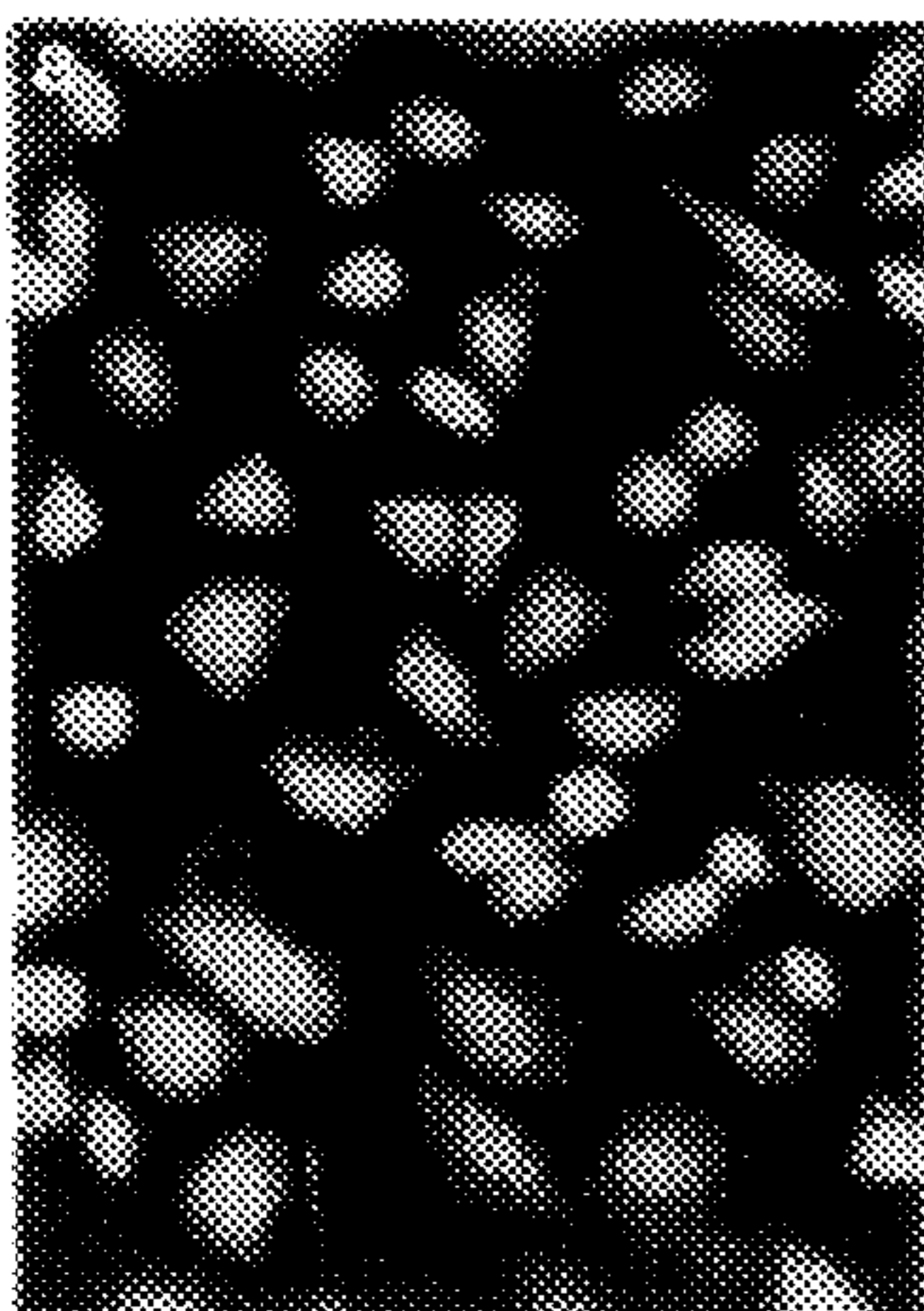


FIG. 12 B



FIG. 12 C



FIG. 13A

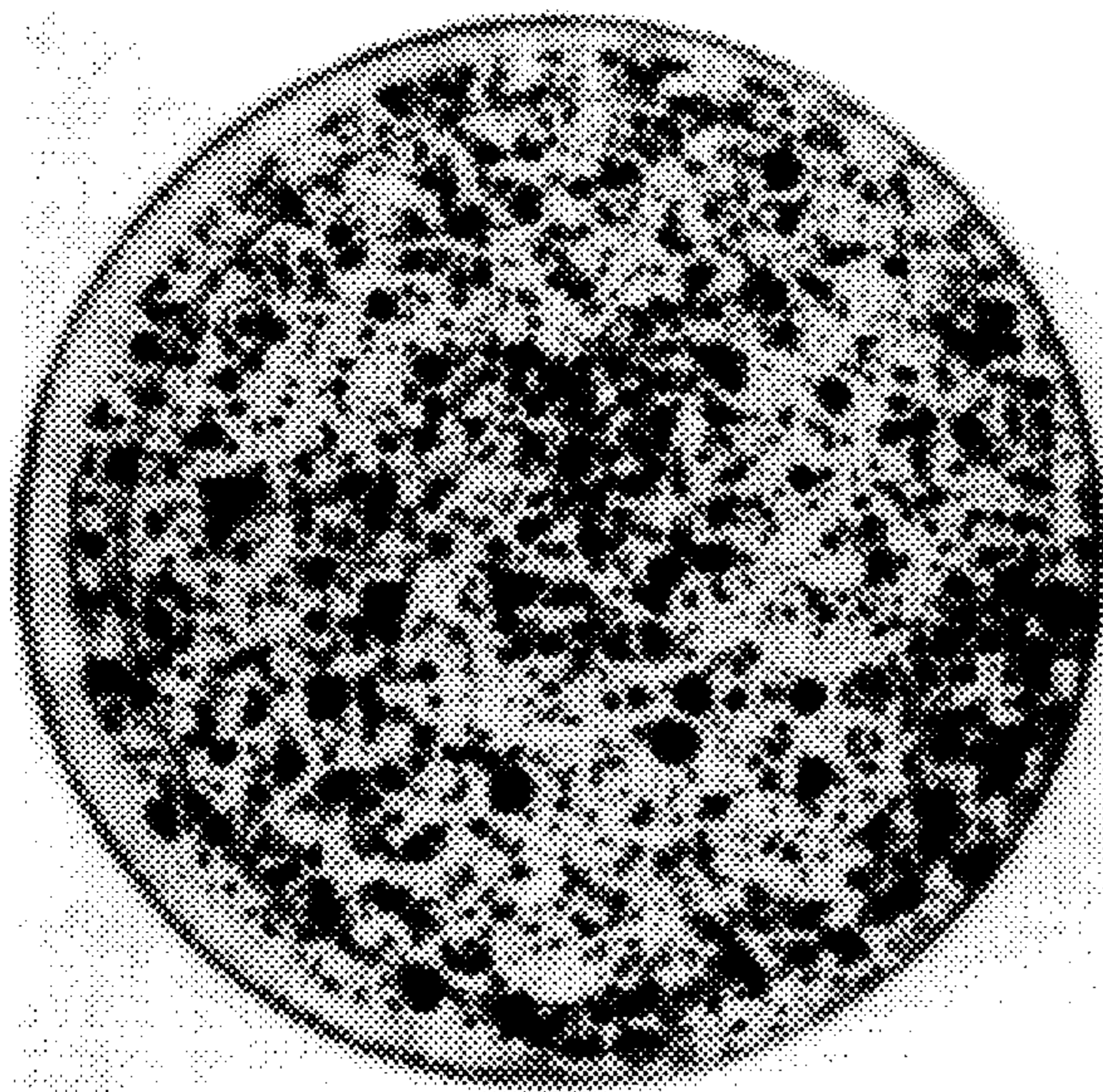


FIG. 13B

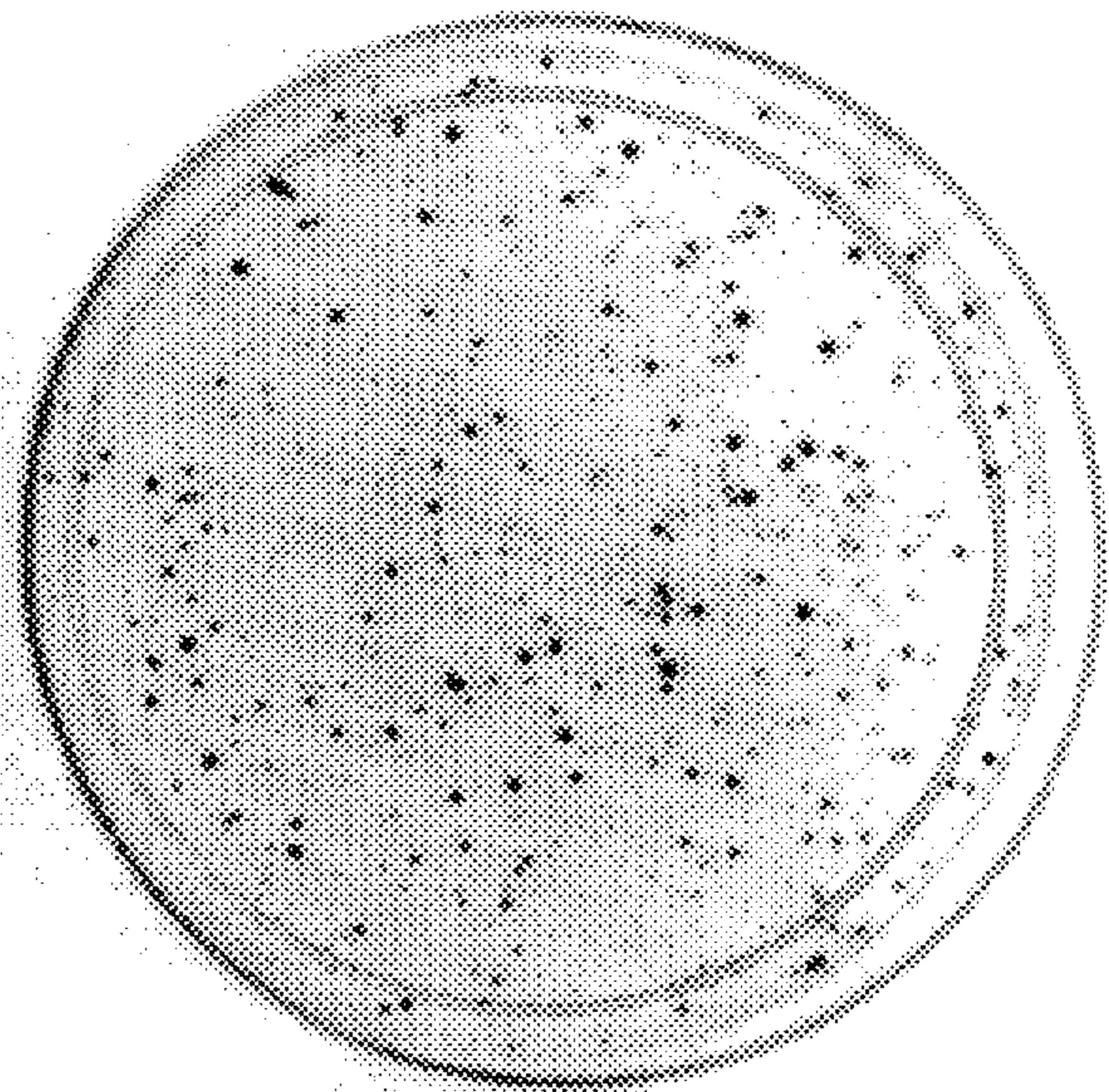


FIG. 14A

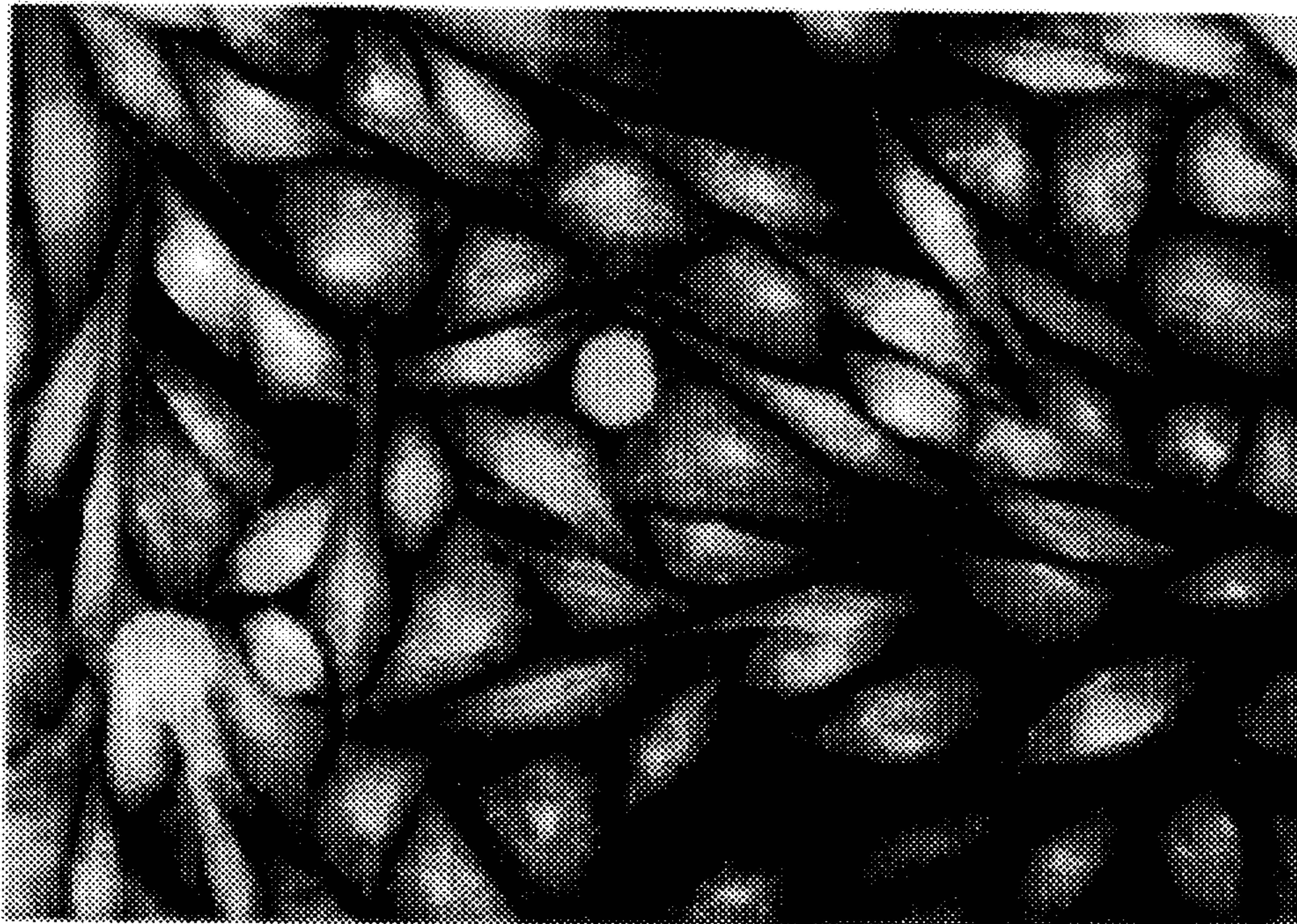
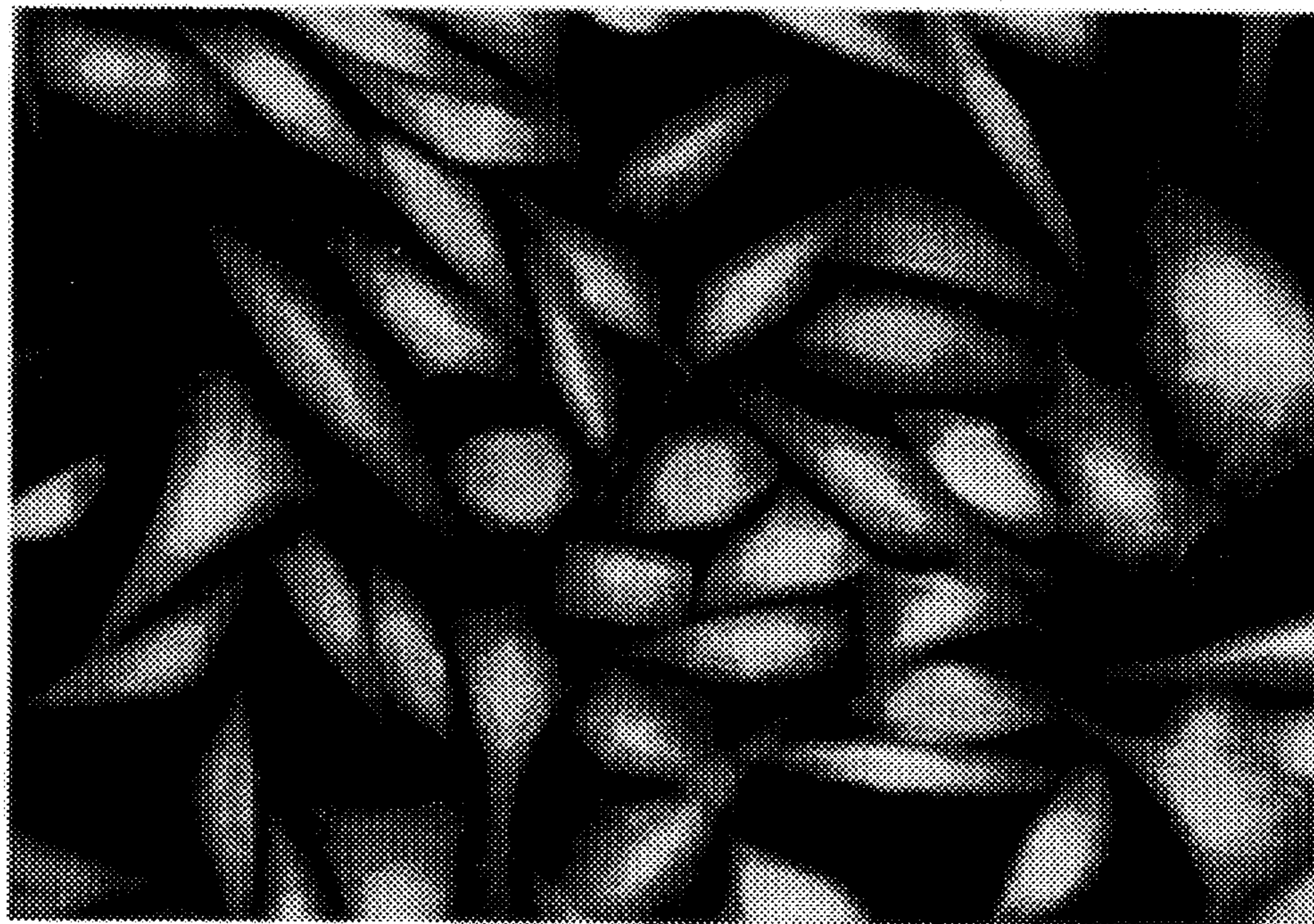


FIG. 14B



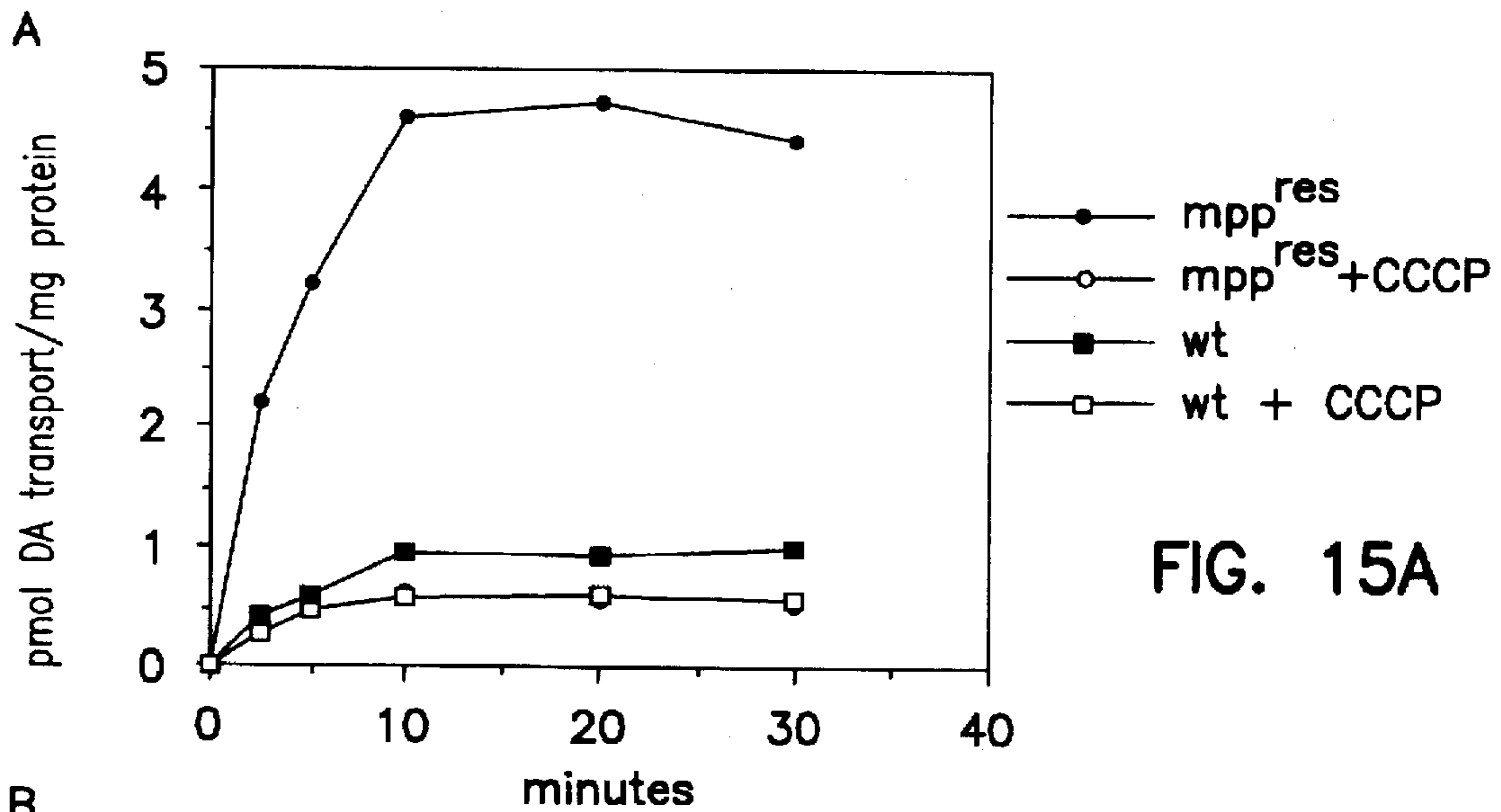


FIG. 15A

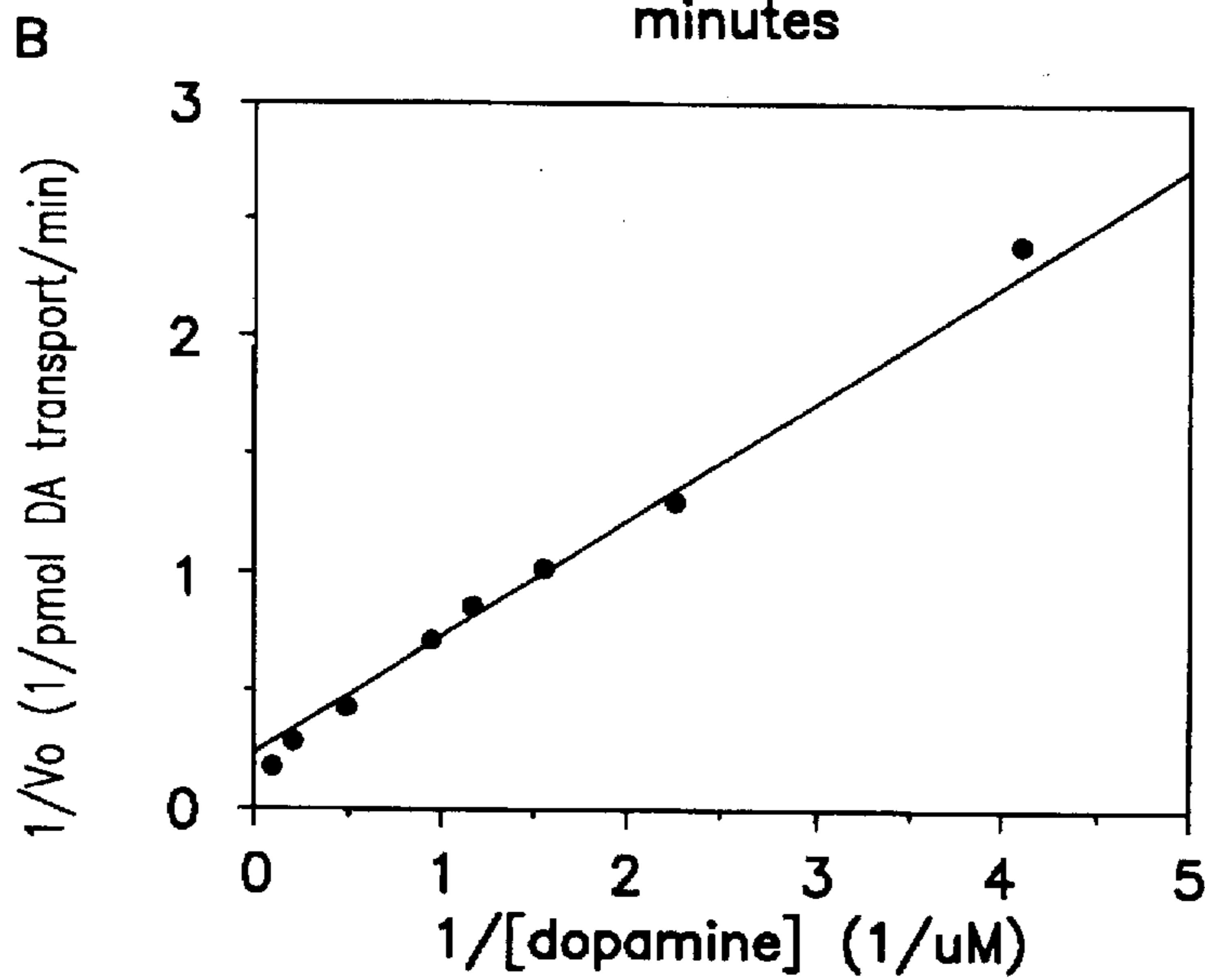


FIG. 15B

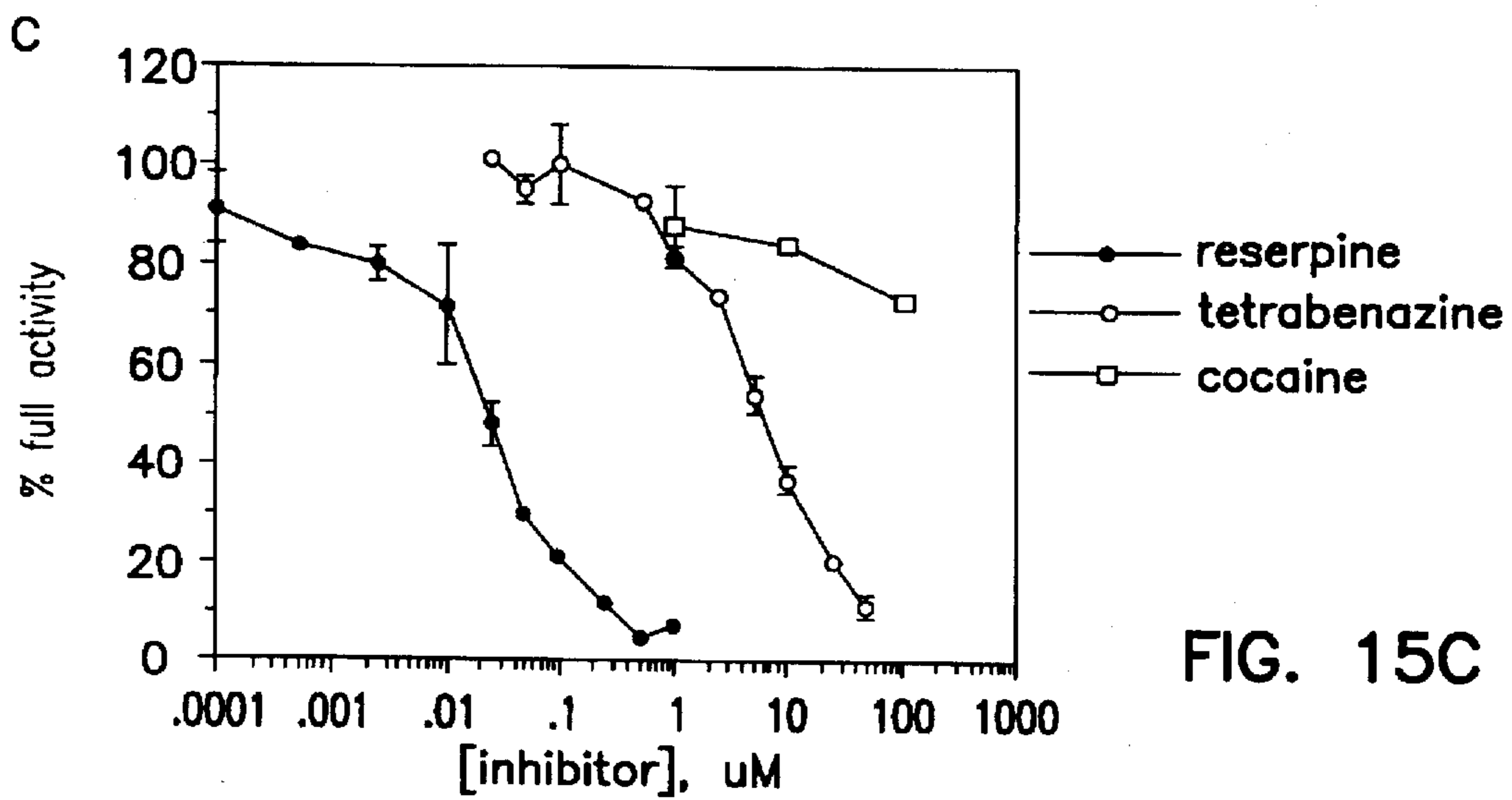


FIG. 15C

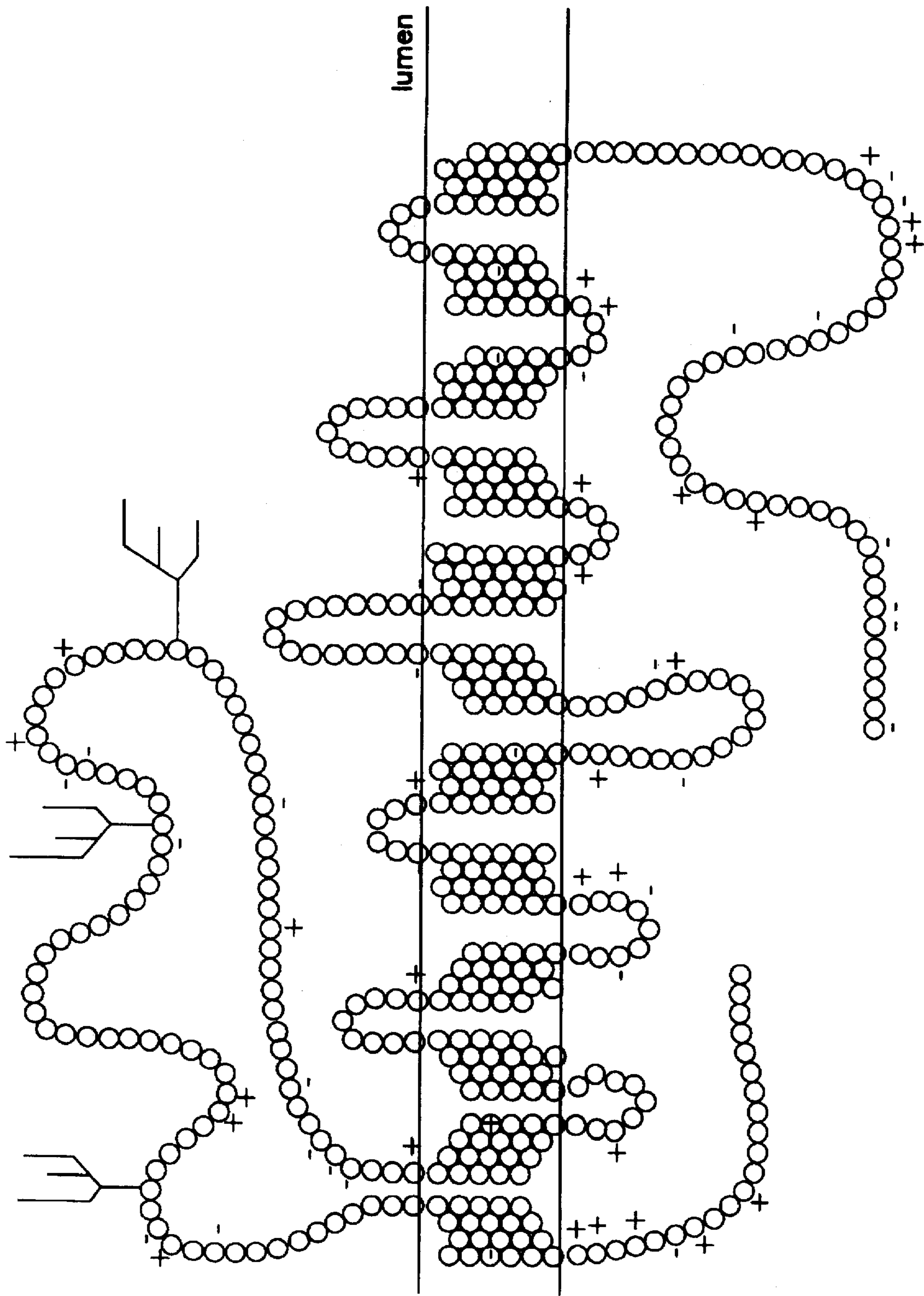


FIG. 16

FIG. 17

```

11
[ SEQ ID NO : 6 ] Mmr taevpaggrrdvpsgVkitalatgfvmatLdVtVvnvagatigeSldttlt.....
[ SEQ ID NO : 7 ] Tet pBR .....mksNnaLiViLgtvtLDavGIGLVmPvLPgLLrDivhSDsias.....
[ SEQ ID NO : 8 ] Tet Tn10 .....mNsstkiaLvitLLDaMGIGLImpVlPTLLrEfiaseDian.....
[ SEQ ID NO : 9 ] BMR .....mekkNitLtIlLtnLFiafLGIGLVIPVtPTImnElhSgt.....
[ SEQ ID NO : 10 ] CGAT rllkegrqsrklvLvVvFvaLLLDnMlltvVVPiVPTFLyatefkDsnsslhrgpsvssq /
[ SEQ ID NO : 11 ] Consensus -----N--L-V-L--LLLD-MGIGLVVPV-PTLL-E---SD-----

```



```

/131
Mmr ....qLtwivdgYvLtfasllmLaGgJanRiGaktVyLwGMgvffLasLacALapTaetL
Tet pBR ....hYGVLLAlyALMQFlcaPvlGaLsDRFGRrpVLLasLLgatidyaiMAttpvlwIL
Tet Tn10 ....hFGVLLAlyALMQvifaPwLgkMsDRFGRrpVLLlsLigasLdyLLLAFssalwML
BMR ....avGyMvAcFAitQLivsPiaGrwvDRFGRkimiviGLLffsvseFLFgigkTveML
CGAT /eenvriGILFaskALMQLlvnPPvGpLtnRiGyhipMFvGFMimflstLMFAFsgTyaLL
Consensus -----GVLLA-YALMQL---P--G-L-DRFGR--VLL-GLL---L--LLFAF--T--ML

```



```

191
Mmr iaarLvqGagaALfmpsslsllVfsfpEkqrRtRmLglwSAivatssglGptVGGLMvs.
Tet pBR YagRivaGitga..tgAvagaYIADiTDgedRaRhFGLMSAcFgvGMVaGPvaGGLLga.
Tet Tn10 YlgRLLsGitga..tgAvaasVIADtTsasqRvkwFGwLgAsFglGLIaGPIIGGFagE.
BMR FitRMLgGisapF.impgvtaFIADiTtiktrpkaLGyMSAaistGFIiGPgIGGFLaE.
CGAT FvaRtLqGigssFssvAglgmLasvyTDnyeRgRaMGialggLaLGLlvGapfGsvMyEf
Consensus ---RLL-GI--AF---A----LIAD-TD---R-R--G-MSA-F--GLI-GP-IGG---E-

```



```

251
Mmr ...afgWesifLLNLpigaigMamtYryiaatEsratrla
Tet pBR IslhaPFLAAvLNGLnlllGcFlMgEshkgerrpmlra
Tet Tn10 IsphsPFFIAALLNivtflvMFwFREtkntrDntdtevg
BMR VnsrlPFFFAAaFaLLaailSiLtLREpernpEngeikgq
CGAT VgkssPFLilAFLaLLdgaLqLciLWpskvspEsamgtsl
Consensus -----PF--AALLNLL---L-MF-LRE-----E-----

```



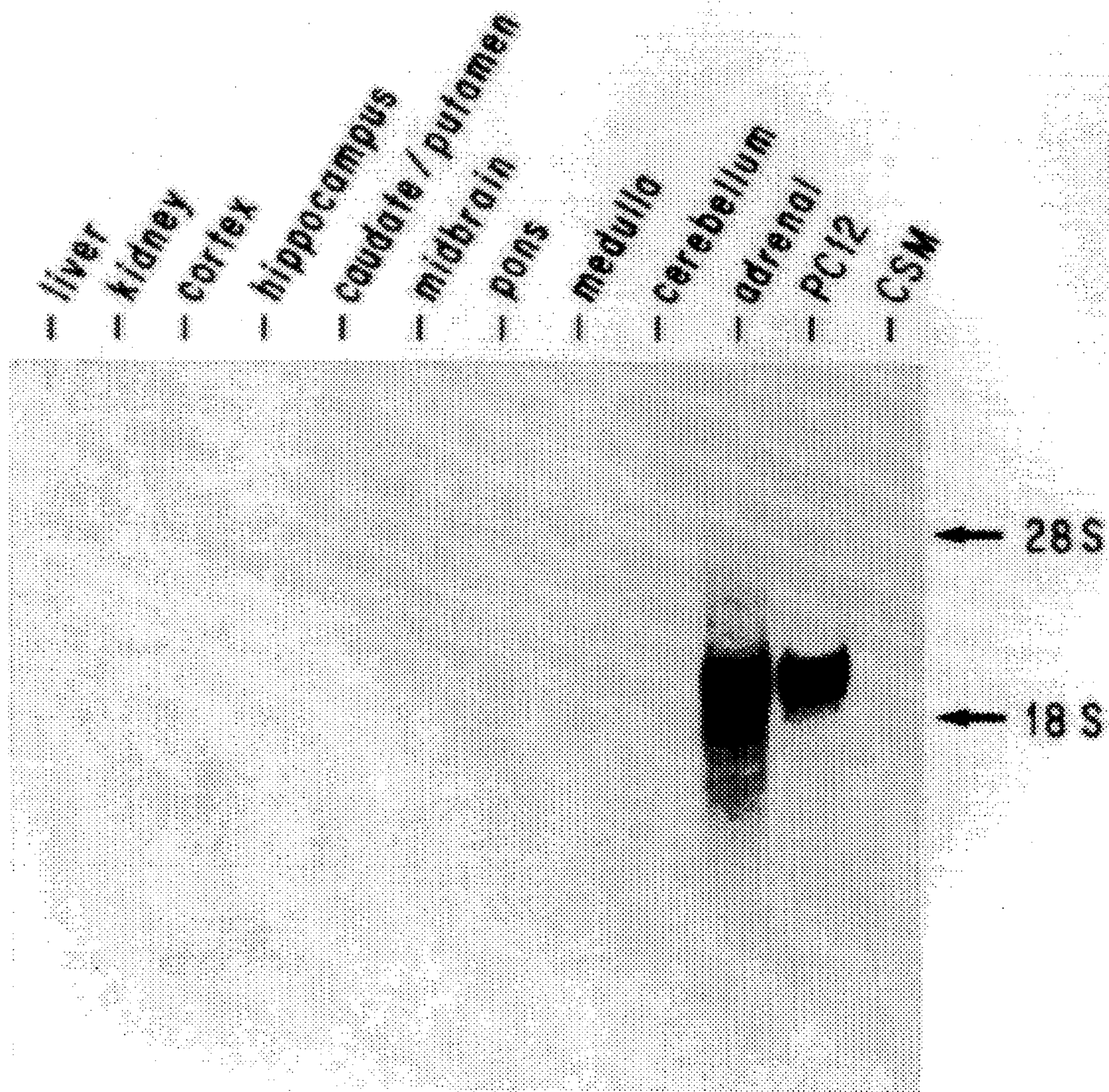


FIG. 18A

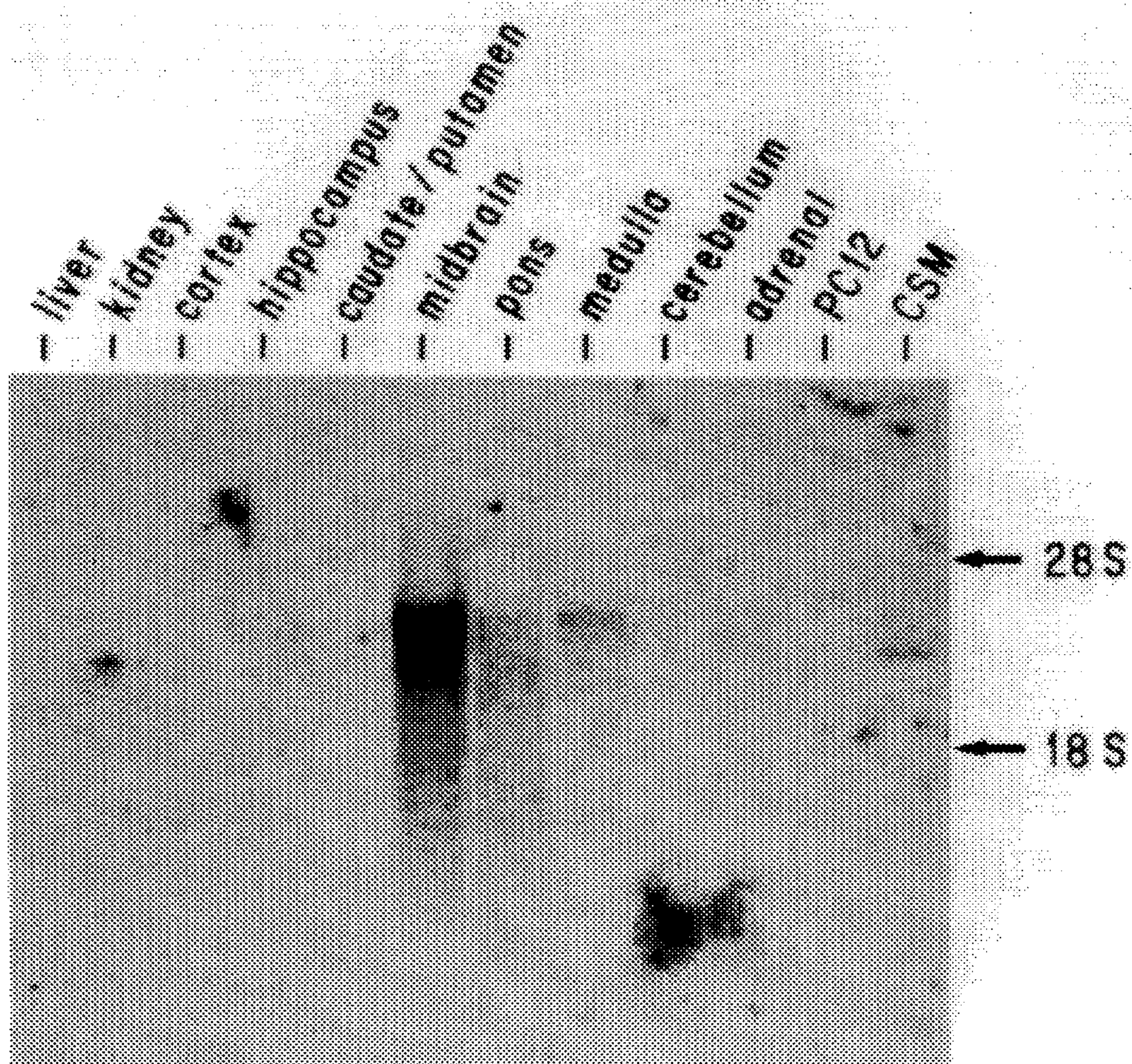


FIG. 18B

FIG. 19A



FIG. 19B



FIG. 19C



FIG. 20

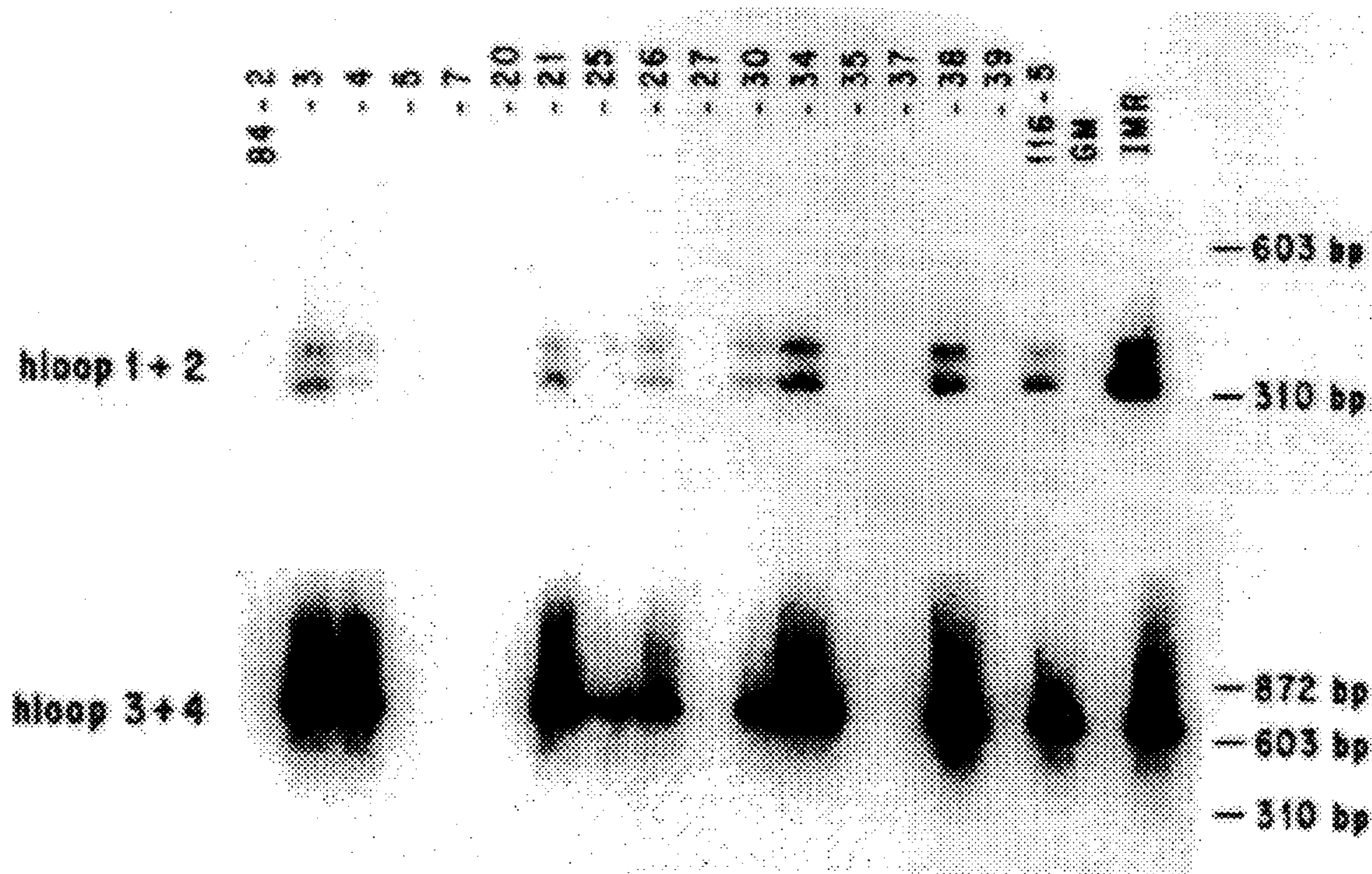


FIG. 21A

[SEQ ID NO : 4] hSVAT MALSeLaL...vRWLqESRrSRKLILFIVFLALLLDNMLLTVVVPIIPSYLY.
[SEQ ID NO : 2] rSVAT MALSDlVL...lRWLRdSRhSRKLILFIVFLALLLDNMLLTVVVPIIPSYLY.
[SEQ ID NO : 13] rCGAT mlqvVLgapqRlLKEgRqSRKlLvVvVFvALLLDNMLLTVVVPIIPSYLYa

┌───────────TM1───────────┐

hSVAT .IKHEKNaT EIQTaRPv htasiSdsfqSIFSYDN ST mvTGNATrd
rSVAT .IKHEKNST EIQTtRP eLv vStS eSIFSYnN STvliTGNATgt
rCGAT tefkdsNSslhrgpsvssqqa Lt SpaFstIFSffDNttTtveehvp..

hSVAT L...TlhqTaTQHmVtNAsaVPSD CPSEDkDLL NENVQVGLLFASKATVQ
rSVAT LpggqshkaTsTQHTVAN.ttVPSD CPSEDxDLL NENVQVGLLFASKATVQ
rCGAT frvtwTngtippvTeAss VPknnC lqgiefLeeENVriGILFASKAlmQ

┌───────────TM2───────────┐

hSVAT LiTNPFIGLLTNRIGYPIPIFAGFCIMFvSTIMFAFSSSYAFLLIARSLOGIG
rSVAT LLTNPFIGLLTNRIGYPIPMFAGFCIMFiSTVMFAFSSSYAFLLIARSLOGIG
rCGAT LLvNPFvGpLTNRIGYhIPMFvGFmIMFlSTLMFAFSgtYAlLfvArtLQIGIG

┌───────────TM3───────────┐

hSVAT SSCSSVAGMGLASVYTDDEERGnvMGIALGGLAMGVLVGPPFGSVLYEFVGK
rSVAT SSCSSVAGMGLASVYTDDEERGkpMGIALGGLAMGVLVGPPFGSVLYEFVGK
rCGAT SSfSSVAGLGLASVYTDNyERGraMGIALGGLALGLLVGaPFGSvmYEFVGK

┌───TM4───┐ ┌───────────TM5───────────┐

hSVAT TAPFLVLAALVLLDGAIQLFVLQPSRVQEPESQKGTPLTLLKDPYILIAAGSI
rSVAT TAPFLVLAALVLLDGAIQLFVLQPSRVQEPESQKGTPLTLLKDPYILIAAGSI
rCGAT ssPFLiLaflaLLDGALQlciLwPSkVSPESamGTslTLLKDPYILVaAGSI

┌───────────TM6───────────┐ ┌───TM7───┐

hSVAT CFANMGIAMLEPALPIWMMETMCSRKWQLGVAFLPASISYLIGTNI FGILAHK
rSVAT CFANMGIAMLEPALPIWMMETMCSRKWQLGVAFLPASISYLIGTNI FGILAHK
rCGAT CLANMGvAiLEttLPIWMMqTMCSPeWQLGIAFLPASvaYLIGTNLFgvLAnK

┌───────────TM8───────────┐

hSVAT MGRWLCALLGMiIVGvSILCIPFAKNIYGLIAPNFGVGFaIGMVDSSMMPIMG
rSVAT MGRWLCALLGMVIVGISILCIPFAKNIYGLIAPNFGVGFaIGMVDSSMMPIMG
rCGAT MGRWLCsLVGMVAVGISlLcVplAhNI fGLIGPNAGlGFAIGMVDSSLMPIMG

┌───────────TM9───────────┐ ┌───────────TM10───────────┐

FIG. 21B

hSVAT YLVDLRHVS^vYGSVYAIADVAFCMGYAIGPSAGGAIAKAIGFPWLMTIIGIID
rSVAT YLVDLRHVS^vYGSVYAIADVAFCMGYAIGPSAGGAIAKAIGFPWLMTIIGIID
rCGAT YLVDLRH^tS^vYGSVYAIADVAFC^vG^fAIGPStGG^vIvq^vIGFPqLMTIIGTIn
┌───────────┴───────────┐ ┌───────────┴───────────┐
 TM11 TM12

hSVAT ILFAPLCFFLRSPPAKEEKMAILMDHNCPIKTKMYT QNNiQSYPIGEDEESESD*
rSVAT IaFAPLCFFLRSPPAKEEKMAILMDHNCPIKTKMYT QNN^vQSYPIGdDEESESD*
rCGAT IiyAPLCcFLqnPPAKEEKrAIL sqeC^PteTqMYTfQkptkafPlGEnsddpSsge*
└

FIG. 22A

hsvatcDNA Folder Alig D Sequence

Sequence Range: 1 to 1898

	10	20	30	40	50	60
[SEQ ID NO : 12]	*	*	*	*	*	*
hsvatcDNA	GGCGCAAGCGACCCCGAGCGGAGCCCGGAGCCATGGCCCTGAGCGAGCTGGCGCTGGTC					

17-3-5 [SEQ ID NO : 3]	50	60	70	80	90
[5008]	C++++GT+ACAGG++++-CC+-GAG+A+++++T++++T++++C+G>				
(rsvatcDNA)					
hsvatcDNA	GGCGCAAGCGACCCCGAGCGGAGCCCGGAGCCATGGCCCTGAGCGAGCTGGCGCTGGTC				

[SEQ ID NO : 1]					T	CA
5-6-1	240	250	260	270	280	
[3040]	A+-C+C+--C+T+-CT+--C++++TT--+++++CT++A+GTT+TT+G++T+++CC+G>					
(rcgatcDNA)						
hsvatcDNA	GGCGCAAGCGACCCCGAGCGGAGCCCGGAGCCATGGCCCTGAGCGAGCTGGCGCTGGTC					

	70	80	90	100	110	120
	*	*	*	*	*	*
hsvatcDNA	CGCTGGCTGCAGGAGAGCCCGCTCGCGGAAGCTCATCCTGTTTCATCGTGTTCCCTGGCG					

17-3-5	100	110	120	130	140	150
[5008]	++A+++++G+++C+++++A+++++C++A++G+++++T+++>					
hsvatcDNA	CGCTGGCTGCAGGAGAGCCCGCTCGCGGAAGCTCATCCTGTTTCATCGTGTTCCCTGGCG					

5-6-1	300	310	320	330	340	350
[3040]	++G+T++++A++++AG+AA+G+AG++C++C++++GG+G+++G+GG+G+++++G++++T>					
hsvatcDNA	CGCTGGCTGCAGGAGAGCCCGCTCGCGGAAGCTCATCCTGTTTCATCGTGTTCCCTGGCG					

FIG. 22B

	130	140	150	160	170	180
	*	*	*	*	*	*
hsvatcDNA	CTGCTGCTGGACAACATGCTGCTCACTGTCGTGGTCCCCATCATCCCAAGTTATCTGTAC					
17-3-5	160	170	180	190	200	210
[5008]	+++++T+++++C+++++T+++++C++C+++++>					
hsvatcDNA	CTGCTGCTGGACAACATGCTGCTCACTGTCGTGGTCCCCATCATCCCAAGTTATCTGTAC					
5-6-1	360	370	380	390	400	410
[3040]	+++++T+++++G+++++G+++++TG+G++C+CC+TC+++++>					

FIG. 22C

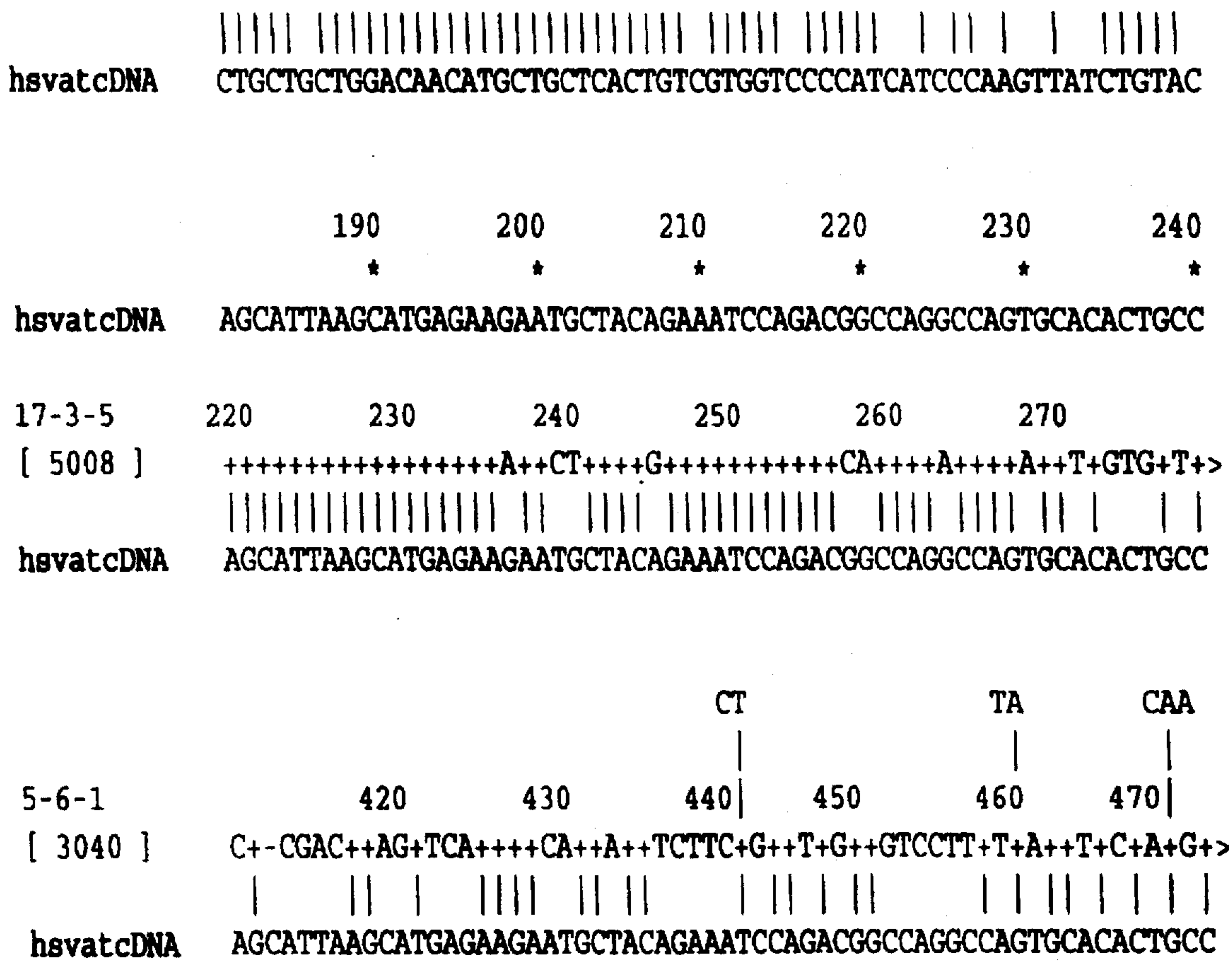


FIG. 22D

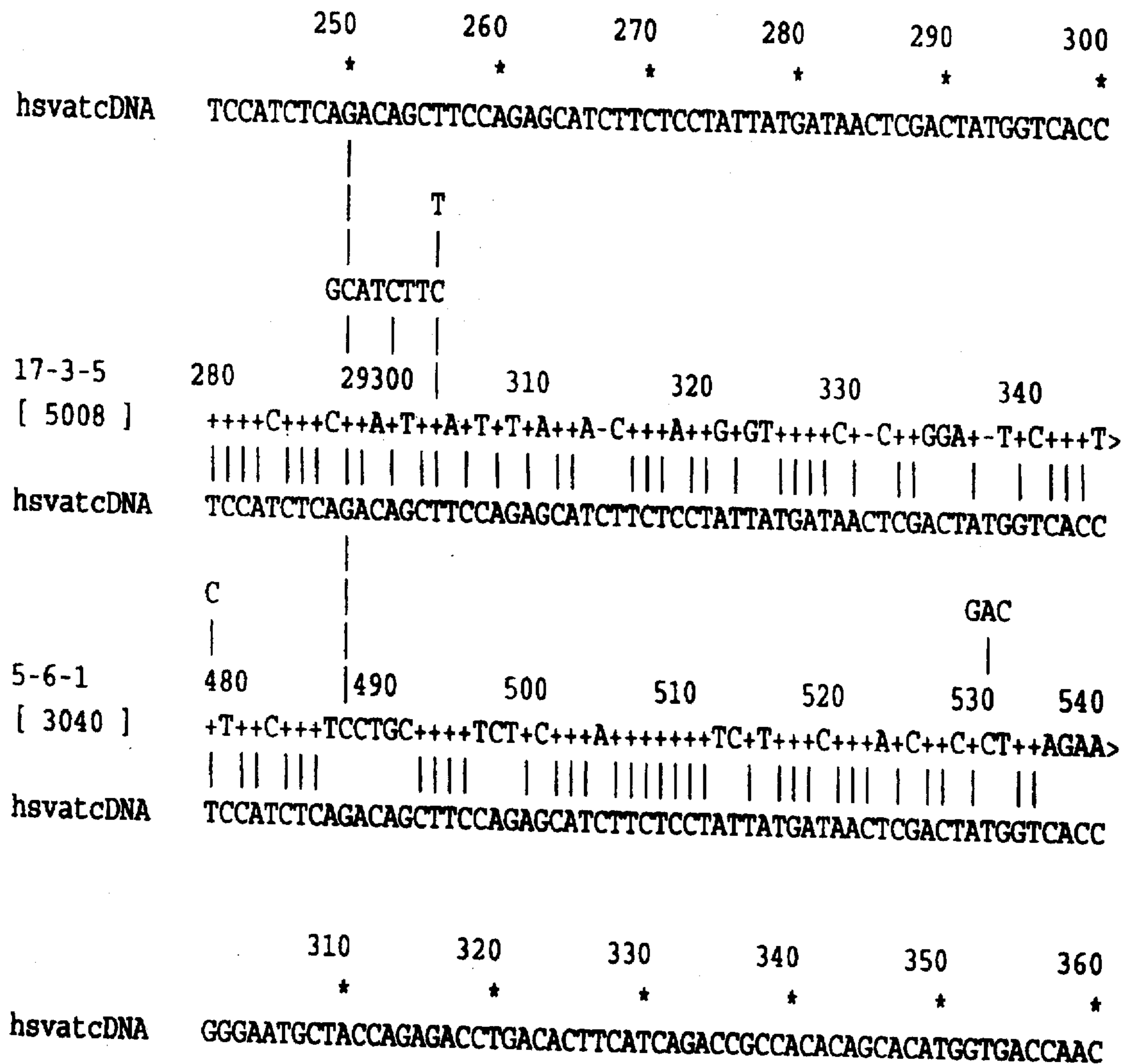


FIG. 22E

17-3-5
[5008]

350 | 360 370 380 390 400

++++C-T++T++G+++GG+A+T+++ACA+GGCT+++AG+++++++CT+++G+T+-->

|||| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

hsvatcDNA GGAATGCTACCAGAGACCTGACACTTCATCAGACCGCCACACAGCACATGGTGACCAAC

5-6-1
[3040]

550 560 570 580 590 600

+AAC+-G+++-CCTTC+G++TA++++GGA++A+TG++AC++TCC+T+CCA++C++TG+A>

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

hsvatcDNA GGAATGCTACCAGAGACCTGACACTTCATCAGACCGCCACACAGCACATGGTGACCAAC

TC
|

hsvatcDNA

370 380 390 400 410 420

* * * * *

GCGTCCGCTGTTCCCTCCGACTGTCCCAGTGAAGACAAAGACCTCCTGAATGAAAACGTG

17-3-5
[5008]

410 420 430 440 450 460

A+-A++A++++C++++G+++++G+++++T+++++G++T+++>

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

hsvatcDNA GCGTCCGCTGTTCCCTCCGACTGTCCCAGTGAAGACAAAGACCTCCTGAATGAAAACGTG

5-6-1
[3040]

610 620 630 640 660

++CAG+T+A++A++AAAA++AACTG+TTGC+++GG+T+++GT++TA+-A+++++T>

|| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

hsvatcDNA GCGTCCGCTGTTCCCTCCGACTGTCCCAGTGAAGACAAAGACCTCCTGAATGAAAACGTG

TAGA
|

FIG. 22F

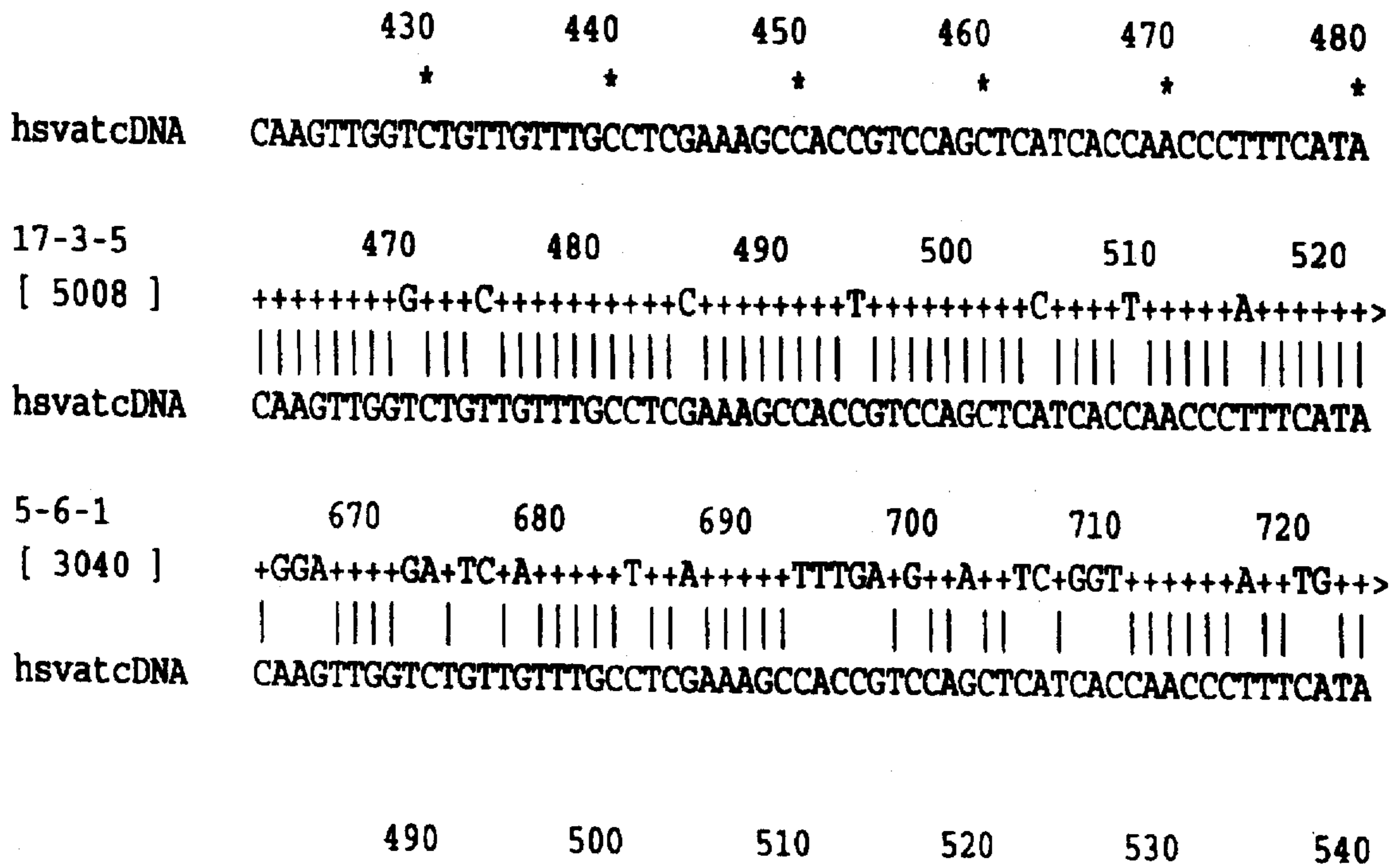


FIG. 22 G

	*	*	*	*	*	*
hsvatcDNA	GGACTACTGACCAACAGAATTGGCTATCCAATTCCCATATTTGCGGGATTCTGCATCATG					
17-3-5	530	540	550	560	570	580
[5008]	++++T++++G++++C++C++++>					
hsvatcDNA	GGACTACTGACCAACAGAATTGGCTATCCAATTCCCATATTTGCGGGATTCTGCATCATG					
5-6-1	730	740	750	760	770	780
[3040]	+++CT++T++T++++G++++AC++C++++G++++TT++C++TATG++++>					
hsvatcDNA	GGACTACTGACCAACAGAATTGGCTATCCAATTCCCATATTTGCGGGATTCTGCATCATG					
	550	560	570	580	590	600
	*	*	*	*	*	*
hsvatcDNA	TTTGTCTCAACAATTATGTTTGCCTTCTCCAGCAGCTATGCCTTCTGCTGATTGCCAGG					
17-3-5	590	600	610	620	630	640
[5008]	+++A++++G++++C++++>					
hsvatcDNA	TTTGTCTCAACAATTATGTTTGCCTTCTCCAGCAGCTATGCCTTCTGCTGATTGCCAGG					
5-6-1	790	800	810	820	830	840
[3040]	+++C+++C++C+A++++T++++TG+++C++++C+G++AT+TG+G+++C+A>					
hsvatcDNA	TTTGTCTCAACAATTATGTTTGCCTTCTCCAGCAGCTATGCCTTCTGCTGATTGCCAGG					
	610	620	630	640	650	660
	*	*	*	*	*	*
hsvatcDNA	TCGCTGCAGGGCATCGGCTCGTCCTGCTCCTCTGTGGCTGGGATGGGCATGCTTGCCAGT					
17-3-5	650	660	670	680	690	700
[5008]	++C++T++++A++T++++C++++A++C++++T++++G++++C>					
hsvatcDNA	TCGCTGCAGGGCATCGGCTCGTCCTGCTCCTCTGTGGCTGGGATGGGCATGCTTGCCAGT					

FIG. 22H

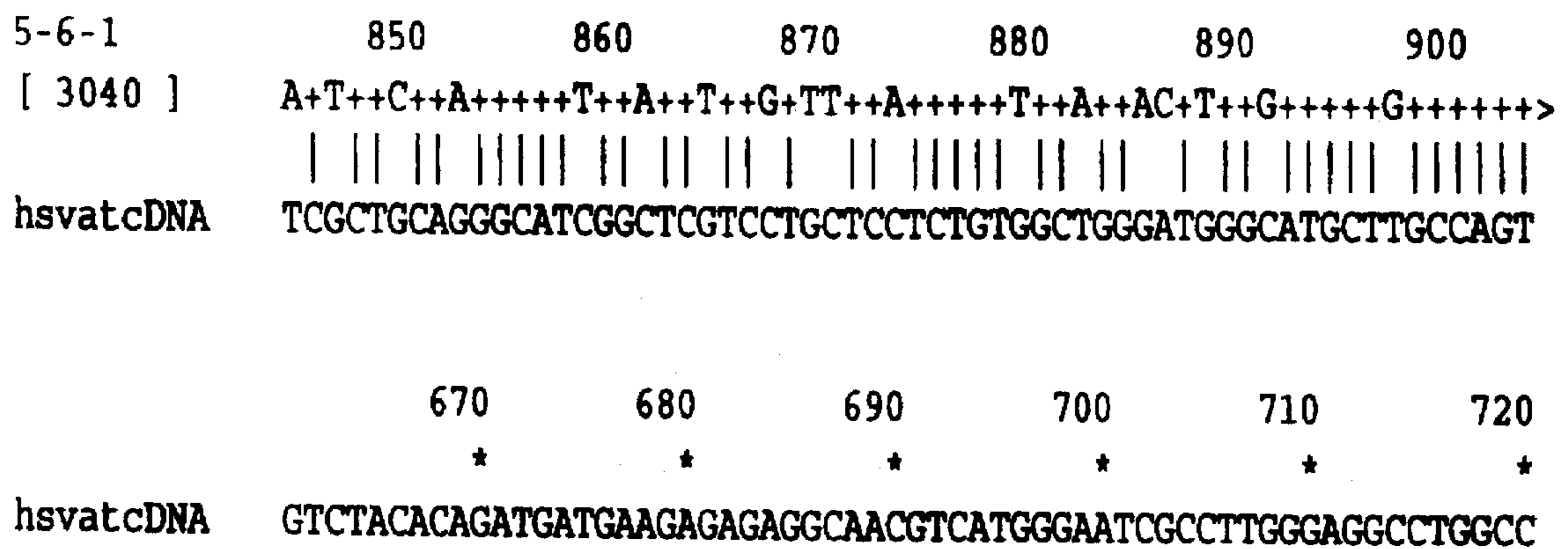


FIG. 22I

17-3-5 710 720 730 740 750 760
 [5008] ++G++++++G++++G++G++GCC+++++C++T++T++++T++++++>
 || ||||| ||||| || || ||||| || || ||||| |||||
 hsvatcDNA GTCTACACAGATGATGAAGAGAGAGGCAACGTCATGGGAATCGCCTTGGGAGGCCTGGCC

5-6-1 910 920 930 940 950 960
 [3040] +++++T++T++CA+CT+T+++++G+GA+C++++++T++T++++G++++++>
 ||||| || | ||||| || ||||| || ||||| ||||| |||||
 hsvatcDNA GTCTACACAGATGATGAAGAGAGAGGCAACGTCATGGGAATCGCCTTGGGAGGCCTGGCC

 730 740 750 760 770 780
 * * * * * *
 hsvatcDNA ATGGGGGTCTTAGTGGGCCCCCCTTCGGGAGTGTGCTCTATGAGTTTGTGGGGAAGACG

17-3-5 770 780 790 | 800 810 820
 [5008] +++++A++++++A++++++A>
 ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
 hsvatcDNA ATGGGGGTCTTAGTGGGCCCCCCTTCGGGAGTGTGCTCTATGAGTTTGTGGGGAAGACG

5-6-1 970 980 990 | 1000 1010 1020
 [3040] T++++AC+TC+G++++AG+A++T++++A+++++A+G++++A+++++C+++T+C>
 ||||| || ||||| || ||||| ||||| ||||| ||||| |||||
 hsvatcDNA ATGGGGGTCTTAGTGGGCCCCCCTTCGGGAGTGTGCTCTATGAGTTTGTGGGGAAGACG

FIG. 22J

	790	800	810	820	830	840
	*	*	*	*	*	*
hsvatcDNA	GCTCCGTTCCGGTGCTGGCCGCCCTGGTACTCTTGGATGGAGCTATTCAGCTCTTTGTG					
17-3-5	830	840	850	860	870	880
[5008]	+++++C+++++A++T++T+++G+++++G+++++>					
hsvatcDNA	GCTCCGTTCCGGTGCTGGCCGCCCTGGTACTCTTGGATGGAGCTATTCAGCTCTTTGTG					
5-6-1	1030	1040	1050	1060	1070	1080
[3040]	T+A++A++++CA+CT++++TT+T+++C+++TC+++++C+C++A++T+GCA+C>					
hsvatcDNA	GCTCCGTTCCGGTGCTGGCCGCCCTGGTACTCTTGGATGGAGCTATTCAGCTCTTTGTG					
	850	860	870	880	890	900
	*	*	*	*	*	*
hsvatcDNA	CTCCAGCCGTCCTGGTGCAGCCAGAGATCAGAAGGGGACACCCCTAACCACGCTGCTG					
17-3-5	890	900	910	920	930	940
[5008]	+++++A++A+++++T++++G++CT++++>					

FIG. 22K

hsvatcDNA CTCCAGCCGTCCCGGGTGCAGCCAGAGAGTCAGAAGGGGACACCCCTAACACGCTGCTG

5-6-1 1090 1100 1110 1120 1130 1140
[3040] ++ATG+++T++GAAA+++TCT++T+++++GCC+T+++++TT+G++TTTG+++++T++C>
|| ||| || ||| || ||||| | ||||| | || ||||| ||
hsvatcDNA CTCCAGCCGTCCCGGGTGCAGCCAGAGAGTCAGAAGGGGACACCCCTAACACGCTGCTG

910 920 930 940 950 960
* * * * *
hsvatcDNA AAGGACCCGTACATCCTCATTGCTGCAGGCTCCATCTGCTTTGCAAACATGGGCATCGCC

17-3-5 950 960 970 980 990 1000
[5008] +++++T++A+++++C+++++G++A+++>
|||| | ||||| ||||| ||||| ||||| ||||| ||||| || |||
hsvatcDNA AAGGACCCGTACATCCTCATTGCTGCAGGCTCCATCTGCTTTGCAAACATGGGCATCGCC

5-6-1 1150 1160 1170 1180 1190 1200
[3040] ++A++++T+++++GG+A++A++++T+++++G++C+++++AG++++>
|| |||| ||||| ||||| | || |||| ||||| || ||||| |||||
hsvatcDNA AAGGACCCGTACATCCTCATTGCTGCAGGCTCCATCTGCTTTGCAAACATGGGCATCGCC

FIG. 22L

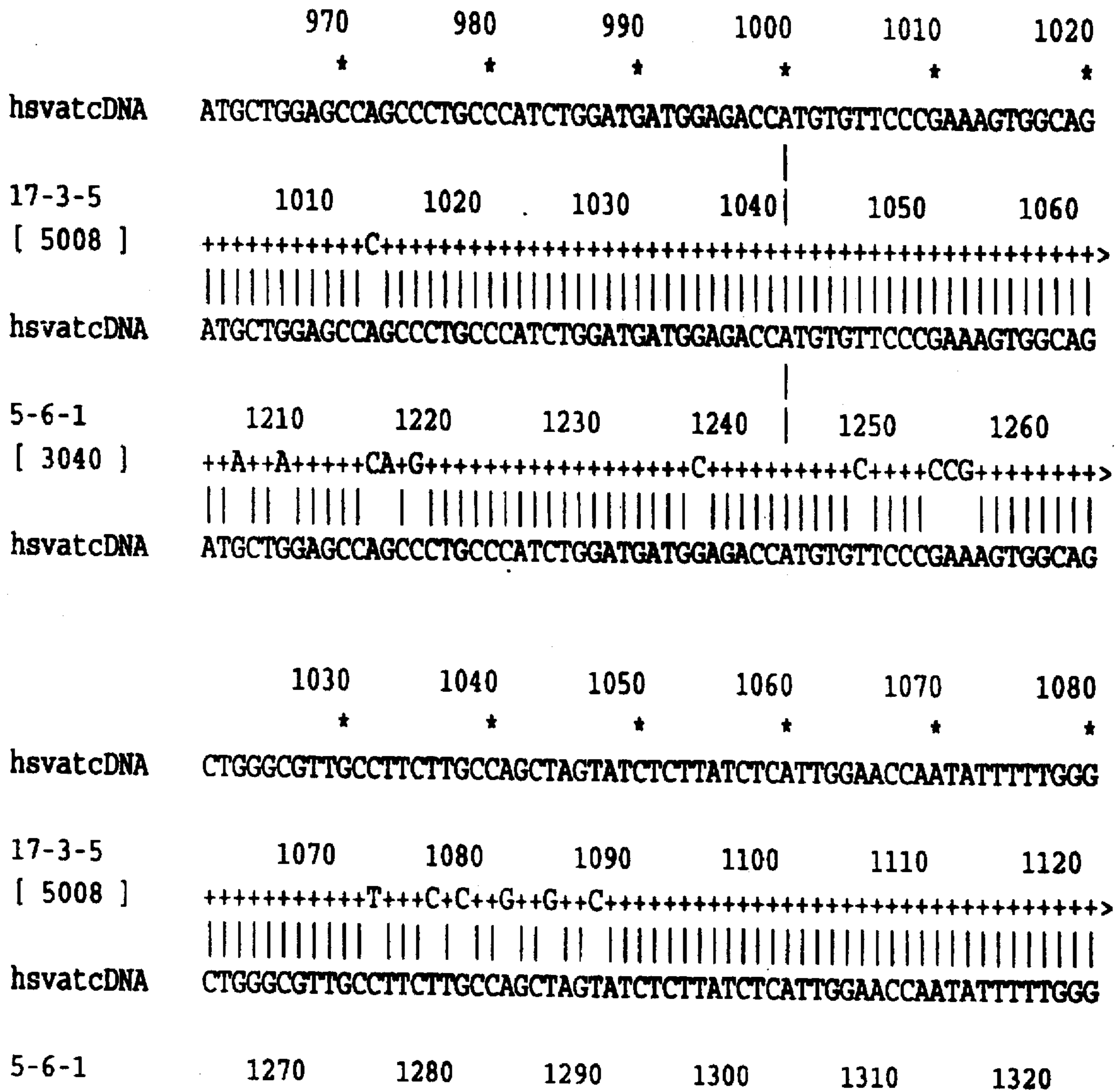


FIG. 22M

```
[ 3040 ]      ++A++TC+G++T+++++++T+++++G+GG+C++C+++++++C++G++CC+C+++++T>
              || || | || ||||| ||||| | | || ||||| || || | |||||
hsvatcDNA     CTGGGCGTTGCCTTCTTGCCAGCTAGTATCTCTTATCTCATTGGAACCAATATTTTGGG
```

```
                1090      1100      1110      1120      1130      1140
                *       *       *       *       *       *
hsvatcDNA     ATACTTGCACACAAAATGGGGAGGTGGCTTTGTGCTCTTCTGGGAATGATAATTGTTGGA
```

```
17-3-5        1130      1140      1150      1160      1170      1180
[ 5008 ]      +++++A+++++A+++++G+++++>
              ||||| ||||| ||||| ||||| ||||| |||||
hsvatcDNA     ATACTTGCACACAAAATGGGGAGGTGGCTTTGTGCTCTTCTGGGAATGATAATTGTTGGA
```

```
5-6-1         1330      1340      1350      1360      1370      1380
[ 3040 ]      G+GT+G++TA+++G+++++TC+++++G++CT+C+++G++T++G+++G+GGCA++A++T>
              | | || |||| ||||| ||||| || | ||| | | ||| | | || |
hsvatcDNA     ATACTTGCACACAAAATGGGGAGGTGGCTTTGTGCTCTTCTGGGAATGATAATTGTTGGA
```

```
                1150      1160      1170      1180      1190      1200
                *       *       *       *       *       *
hsvatcDNA     GTCAGCATTTTATGTATTCCATTTGCAAAAAACATTTATGGACTCATAGCTCCGAAC TTT
```

```
17-3-5        1190      1200      1210      1220      1230      1240
[ 5008 ]      A+++++C++C++C+++++++T++C+++++++C++++C+++++>
              ||||| ||||| || || ||||| || ||||| ||||| |||||
hsvatcDNA     GTCAGCATTTTATGTATTCCATTTGCAAAAAACATTTATGGACTCATAGCTCCGAAC TTT
```

```
5-6-1         1390      1400      1410      1420      1430      1440
[ 3040 ]      A++++T+GC+C+++G+A++TC+G++TC+C++T+++T++T++T+GC++C++TGCA>
              |||| | | ||| | || | || | ||| ||| ||| | ||| ||
hsvatcDNA     GTCAGCATTTTATGTATTCCATTTGCAAAAAACATTTATGGACTCATAGCTCCGAAC TTT
```

FIG. 22N

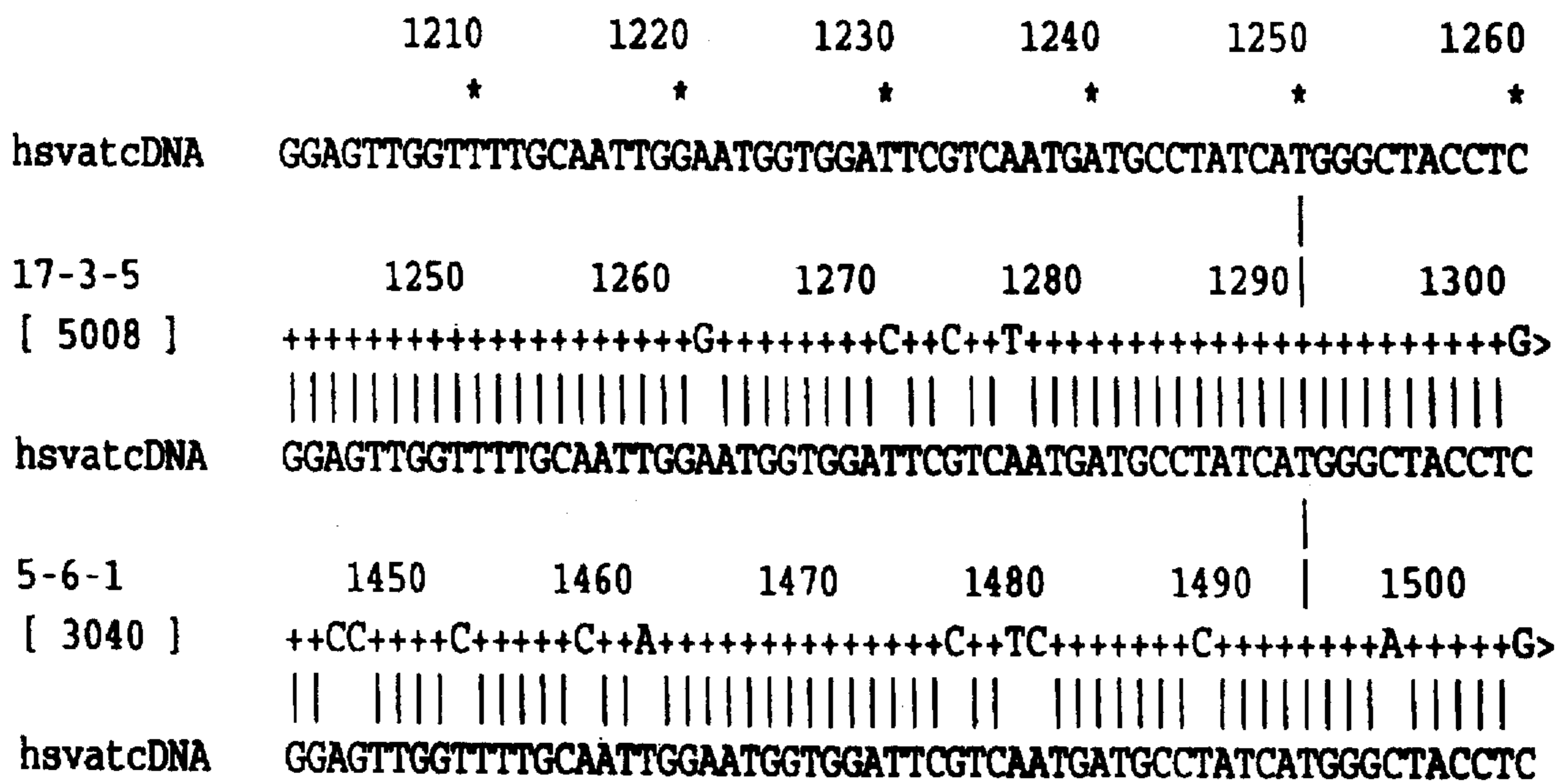


FIG. 22 O

	1270	1280	1290	1300	1310	1320
	*	*	*	*	*	*
hsvatcDNA	GTAGACCTGCGGCACGTGTCCGTCTATGGGAGTGTGTACGCCATTGCGGATGTGGCATT					
17-3-5	1310	1320	1330	1340	1350	1360
[5008]	++T+++++T++++T+++++T++T+++++A++C++++C+++>					
hsvatcDNA	GTAGACCTGCGGCACGTGTCCGTCTATGGGAGTGTGTACGCCATTGCGGATGTGGCATT					
5-6-1	1510	1520	1530	1540	1550	1560
[3040]	++G++T+A++C++ACC++T++G+++++C++T++++C++C+++++C+++>					
hsvatcDNA	GTAGACCTGCGGCACGTGTCCGTCTATGGGAGTGTGTACGCCATTGCGGATGTGGCATT					
	1330	1340	1350	1360	1370	1380
	*	*	*	*	*	*
hsvatcDNA	TGTATGGGGTATGCTATAGGTCCTTCTGCTGGTGGTGCTATTGCAAAGGCAATTGGATT					
17-3-5	1370	1380	1390	1400	1410	1420
[5008]	+++++C+++++C++++C+++++C++C+++++C+++>					
hsvatcDNA	TGTATGGGGTATGCTATAGGTCCTTCTGCTGGTGGTGCTATTGCAAAGGCAATTGGATT					
5-6-1	1570	1580	1590	1600	1610	1620
[3040]	+++G+++C+T+++++T++C++A+++A+++G+++T+++C+T+C+++TC++++C+++>					
hsvatcDNA	TGTATGGGGTATGCTATAGGTCCTTCTGCTGGTGGTGCTATTGCAAAGGCAATTGGATT					

FIG. 22 P

	1390	1400	1410	1420	1430	1440
	*	*	*	*	*	*
hsvatcDNA	CCATGGCTCATGACAATTATTGGGATAATTGATATTCTTTTGGCCCTCTCTGCTTTTTT					
17-3-5	1430	1440	1450	1460	1470	1480
[5008]	++T++++T+++++CGC++++T+A+++++C>					
hsvatcDNA	CCATGGCTCATGACAATTATTGGGATAATTGATATTCTTTTGGCCCTCTCTGCTTTTTT					
5-6-1	1630	1640	1650	1660	1670	1680
[3040]	++T+++++GTC++C++++T+CC++CA+C++CA+++A+++T+++++GC++C>					
hsvatcDNA	CCATGGCTCATGACAATTATTGGGATAATTGATATTCTTTTGGCCCTCTCTGCTTTTTT					
	1450	1460	1470	1480	1490	1500

FIG. 22Q

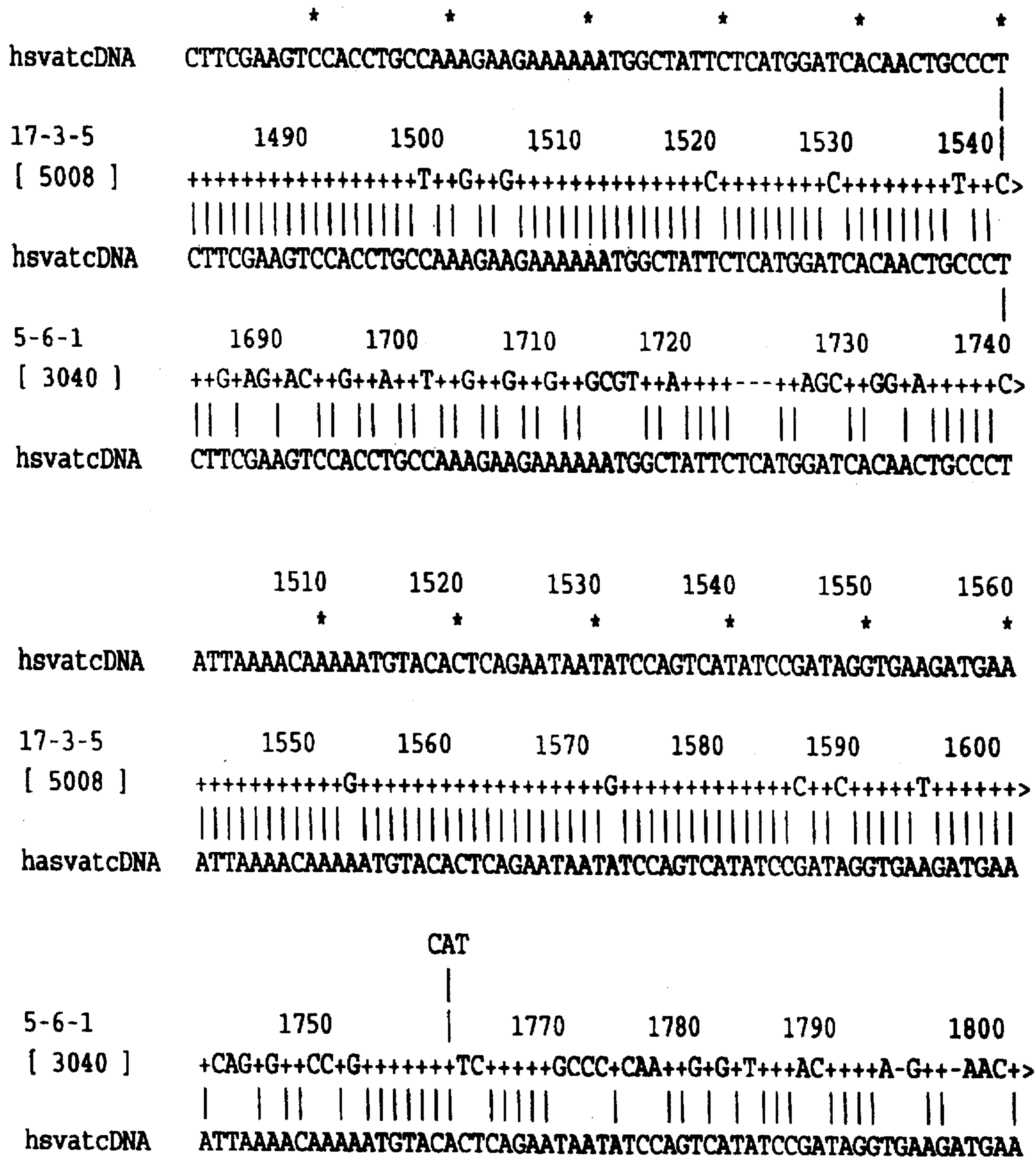


FIG. 22R

	1570	1580	1590	1600	1610	1620
	*	*	*	*	*	*
hsvatcDNA	GAATCTGAAAGTGACTGAGATGAGATCCTCAAAAATCATCAAAGTGTTTAATTGTATAAA					

17-3-5	1610	1620	1630
[5008]	+++++-----C++++C+++T++CG+>		
hsvatcDNA	GAATCTGAAAGTGACTGAGATGAGATCCTGAAAAT		

5-6-1	1810	1820	1830	1840	1850
[3040]	-GCGA+++TCC+AG+A+C+GG+++TAA++GCGG+GGCGAT+TC+-GAGCC+CAC+-TCT>				
hsvatcDNA	GAATCTGAAAGTGACTGAGATGAGATCCTCAAAAATCATCAAAGTGTTTAATTGTATAAA				

1630	1640	1650	1660	1670	1680
*	*	*	*	*	*

FIG. 22S

hsvatcDNA	ACAGTGTTTCCAGTGACACA	ACTCATCCAGA	ACTGTCTTAGTCATACC	CATCCATCCCTGG			
5-6-1	1860	1870	1880	1890	1900	1910	
[3040]	+++G-GGA++++-TCT++T++AG++T++AT+A-T+T+C+C-TT+C+TC+-C-T++A++>						
hsvatcDNA	ACAGTGTTTCCAGTGACACA	ACTCATCCAGA	ACTGTCTTAGTCATACC	CATCCATCCCTGG			
		1690	1700	1710	1720	1730	1740
		*	*	*	*	*	*
hsvatcDNA	TGAAAGAGTAAAACCAA	AGGTTATTATTTCTTTCCAT	GGTTATGGTCGATTGCCAACAG				
5-6-1	1920	1930	1940	1950	1960		
[3040]	CC+CT+CC+TCCT+-CCTTC++++GA+A++++++T+TAC+CACC+GT+AGTG+++CC>						
hsvatcDNA	TGAAAGAGTAAAACCAA	AGGTTATTATTTCTTTCCAT	GGTTATGGTCGATTGCCAACAG				
		1750	1760	1770	1780	1790	1800
		*	*	*	*	*	*
hsvatcDNA	CCTTATAAAGAAAAGA	GCTTTTCTAGGGGTTTG	TATAAATAGTGTGAA	ACTTTATT			
			TGACACC		T		
5-6-1	970	1980	1992000	2010	2020	2030	
[3040]	+ACC+CTCTCCCTCT-GT+++AC+C+T++CCCAC+T+TTG-T+G+AG++C+GGC++++>						
hsvatcDNA	CCTTATAAAGAAAAGA	GCTTTTCTAGGGGTTTG	TATAAATAGTGTGAA	ACTTTATT			

FIG. 22T

	1810	1820	1830	1840	1850	1860
	*	*	*	*	*	*
hsvatcDNA	TATGTATTTAATTTTATTAAATATCATACAATATATTTTGATGAAATAGGTATTGTGTAA					
5-6-1	2040	2050	2060	2070	2080	2090
[3040]	CC+--GCCAGGCCAA+GCG++GC+G++TA++GC+GAG++-G+++C-A++T+C+GCAAGGG>					
hsvatcDNA	TATGTATTTAATTTTATTAAATATCATACAATATATTTTGATGAAATAGGTATTGTGTAA					
	1870	1880	1890			
	*	*	*			
hsvatcDNA	ATCTATAAATATTTGAATCCAAACCAAATATAATTTCC					
5-6-1	2100	2110	2120			
[3040]	G+GAC+C+C+TCC++C+GG++GGA+TG-A+C+++G+G>					
hsvatcDNA	ATCTATAAATATTTGAATCCAAACCAAATATAATTTCC					

FIG. 23



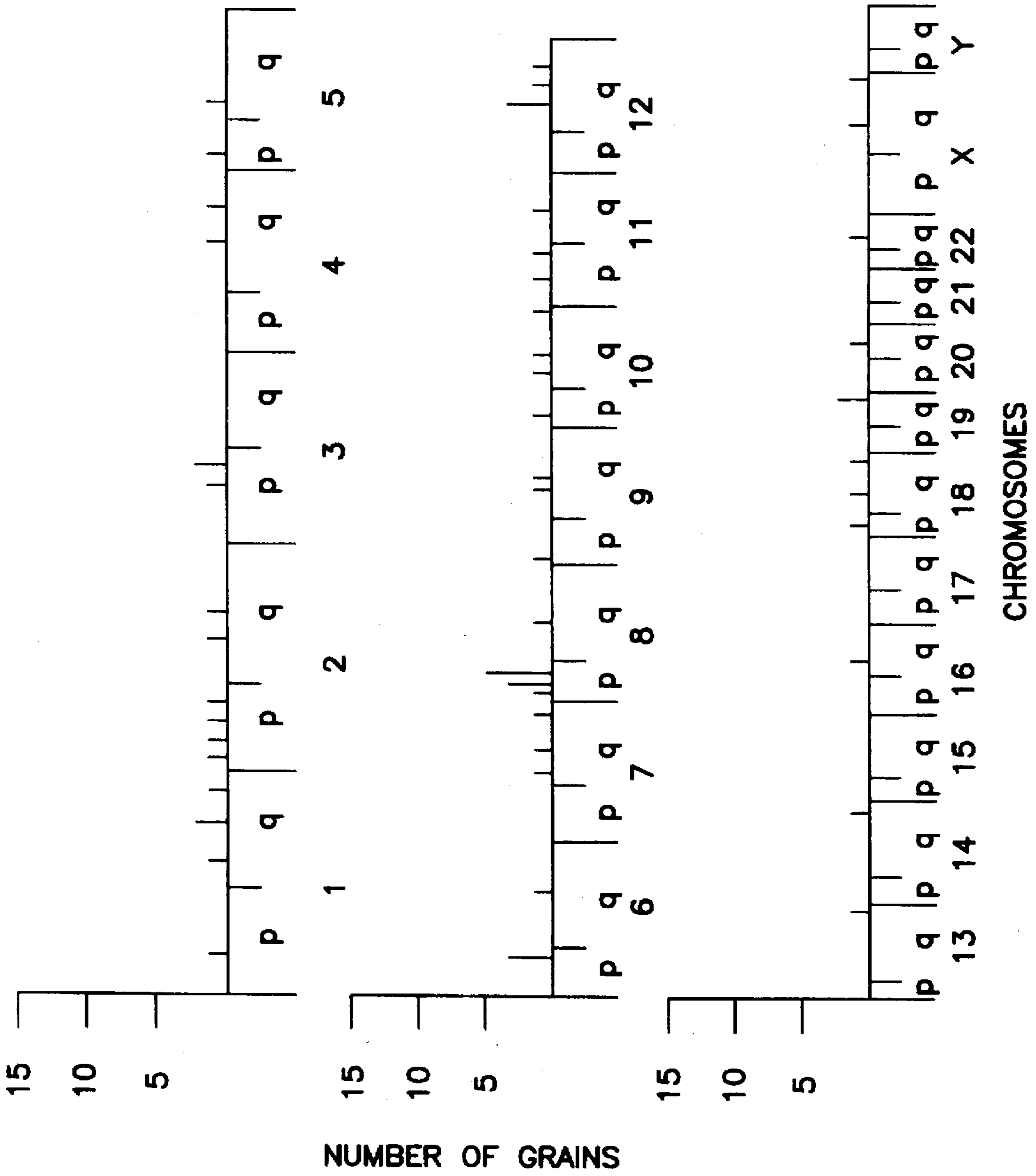
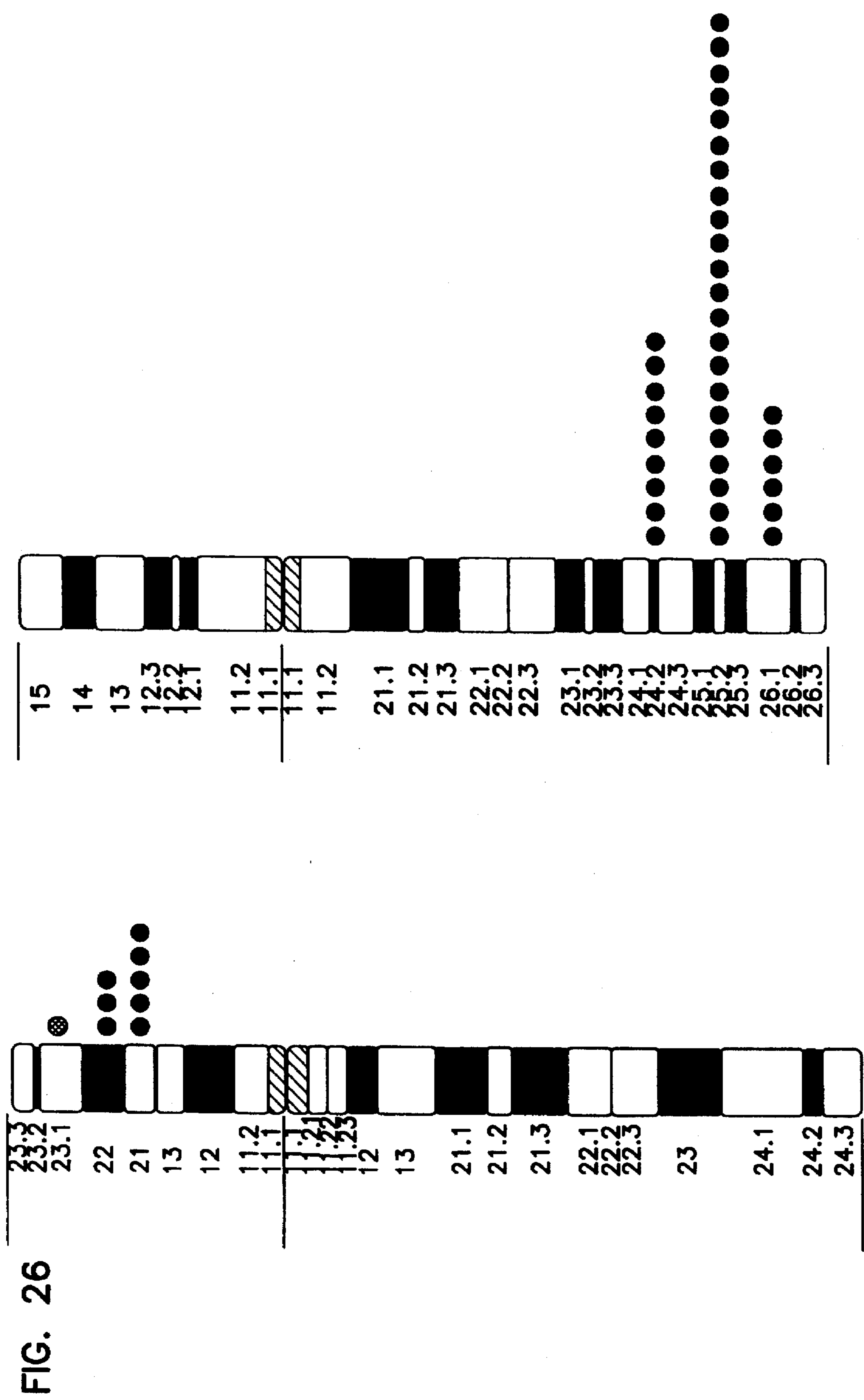


FIG. 25





VESICLE MEMBRANE TRANSPORT PROTEINS

CROSS-REFERENCES

This application is a continuation-in-part of U.S. application Ser. No. 07/923,096 by Robert H. Edwards, filed Jul. 30, 1992, and entitled "Vesicle Membrane Transport Proteins", now abandoned, which in turn is a continuation-in-part of U.S. application Ser. No. 07/899,074 by Robert H. Edwards, filed Jun. 11, 1992 and also entitled "Vesicle Membrane Transport Proteins", now abandoned. Both of these applications are incorporated herein by this reference.

GOVERNMENT RIGHTS

This invention was made with governmental support under National Science Foundation (NSF) grant number BNS 90-11993. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

The present invention relates to the identification, production and manipulation of vesicle membrane transport proteins, and more particularly to the mammalian vesicle amine transport proteins, and to methods of using the vesicle transport proteins to treat Parkinson's disease and other neurological and psychiatric disorders and to screen for drugs that modulate the activity of the transport proteins.

Vesicle membrane transport proteins are membrane-bound proteins that actively transport substances into vesicles such as synaptic vesicles within cells. Vesicle transport activities have been identified for the amines, acetylcholine, gamma-aminobutyric acid (GABA), glycine and glutamate (Burger et al., *Neuron* 7(2):287-93 (1991); Hell et al., *J. Biol. Chem.* 265(4):2111-7 (1990)); Maycox et al., *J. Biol. Chem.* 263(30):15423-8 (1988); Hicks et al., *J. Neurochem.* 57(2):509-19 (1991); Kish et al., *Proc. Natl. Acad. Sci. USA* 86:3877-3881 (1989); Carlson et al., *J. Biol. Chem.* 264:7369-7376 (1989) and Stern-Bach et al., *J. Biol. Chem.* 265:3961-3966 (1990)). Most activities appear to depend on an electrochemical gradient across the vesicle that is generated by the vesicular H⁺-ATPase. Several of these activities have been reconstituted in lipid vesicles, but only the chromaffin granule amine transporter has been purified to any extent (Stern-Bach et al., supra). Binding of [³H]reserpine to this transporter has suggested a molecular weight of 80 kilodaltons (Kd) but no cDNA clone has been made available for this transporter or any other neurotransmitter transporter.

It has been proposed that a neurotoxic insult removes a portion of dopamine neurons and results in the appearance of Parkinsonian symptoms when the dopamine cell population and function decrease below a threshold level (Calne and Langston, *Lancet* ii, 1457-1459 (1983)). Damage to central dopamine neurons in primates (Langston et al., *Science* 219:979-980 (1983); and Burns et al., *Proc. Natl. Acad. Sci. USA* 80:4546-4550 (1983)) and to dopamine nerve terminals in mice (Heikkila et al., *Science* 224:1451-1452 (1984)) caused by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been suggested as a model of Parkinson's disease. The development of MPTP neurotoxicity requires its conversion to the active metabolite 1-methyl-4-phenylpyridinium (MPP⁺) by the B isozyme of monoamine oxidase (Chiba et al., *Biochem. Biophys. Res. Commun.* 120:574-578 (1984); Heikkila, supra; Langston et al., *Science* 225:1480-1482 (1984); and

Markey et al., *Nature* 311:464-466 (1984)). Dopaminergic cells of the substantia nigra then accumulate high levels of MPP⁺ by uptake through the high-affinity plasma membrane catecholamine transporter (Javitch et al., *Proc. Natl. Acad. Sci. USA* 82:2173-2177 (1985); and Snyder et al. in MPTP: A Neurotoxin Producing a Parkinsonian Syndrome, (eds. Markey et al., Academic Press, New York), pp. 191-201 (1986)). Inside the cell, MPP⁺ enters mitochondria (Ramsay and Singer, *J. Biochem.* 261:7585-7587 (1986)) and inhibits respiration at the level of complex I, apparently by binding near the site of action of the other mitochondrial toxins rotenone and piericidin (Krueger et al., *Biochem. Biophys. Res. Commun.* 169:123-128 (1990); and Ramsay et al., *J. Neurochem.* 56:1184-1190 (1991)).

Because MPTP reproduces the pattern of neuronal degeneration observed in Parkinson's disease (PD), this form of toxicity has been used as a model system to dissect the cellular components responsible for selective neuronal vulnerability in the idiopathic human disease. Studies of patients with Parkinson's disease support the utility of MPTP as a model for PD. Both brain tissue and circulating platelets from patients with PD show a selective reduction in the same mitochondrial component affected by MPTP, respiratory complex I (Mizuno et al., *Biochem. Biophys. Res. Commun.* 163:1450-1455 (1989); Parker et al., *Ann. Neurol.* 26:719-723 (1989) and Shoffner et al., *Ann. Neurol.* 30:332-339 (1991)). Deprenyl, which inhibits the enzyme that activates MPTP, monoamine oxidase, also appears to slow the rate of progression in idiopathic PD (Parkinson Study Group, *New Engl. J. Med.* 321:1364-1371 (1989)). However, Parkinsonism induced by MPTP develops over days to weeks whereas idiopathic PD develops over years. Explanations for the relatively slow rate of progression in idiopathic PD include chronic, low levels of exposure to an environmental toxin similar to MPTP, oxidative stress related to the cytoplasmic accumulation of dopamine, or the trapping of free radicals by deposited dopamine and lipofuscin that predisposes these cells to mitochondrial injury (Cohen, *J. Neural Transmission Suppl.* 32:229-238 (1990)).

The MPTP model has led to the identification of multiple pathogenetic factors that may also contribute to idiopathic Parkinson's disease. The amelioration of toxicity with an excitatory amino acid antagonist suggests that neural excitation plays a role in the injury produced by MPP⁺ in vivo (Turski et al., *Nature* 349:414-418 (1991)). In addition, neurotrophic factors such as brain-derived neurotrophic factor (BDNF) can protect midbrain cells from MPP⁺ in vitro (Hyman et al., *Nature* 350:230-232 (1991)). However, accumulation of the toxin through a high-affinity plasma membrane catecholamine transporter is required for susceptibility to MPTP in vivo. The expression of this transport activity accounts for the selective cell vulnerability observed in both the MPTP model and idiopathic PD, with several notable exceptions.

Adrenal chromaffin cells and postganglionic sympathetic neurons express a high-affinity catecholamine uptake system, but unlike dopaminergic neurons in the midbrain, do not degenerate in response to MPTP or in most cases of idiopathic PD. Chromaffin cells have even been demonstrated to accumulate [³H]-MPP⁺ but with relatively little toxicity. (Reinhard et al., *Proc. Natl. Acad. Sci. USA* 84:8160-8164 (1987)). Reinhard et al. proposed that the selective resistance of adrenal medulla cells to MPP⁺ was due to subcellular compartmentalization of the neurotoxin within catecholamine storage vesicles. These researchers demonstrated that when chromaffin cells were incubated with MPP⁺ in the presence of tetrabenazine, an inhibitor of

vesicular uptake, that chromaffin cell toxicity was potentiated. They proposed that the relative resistance of some brain monoaminergic neurons to the toxic actions of MPTP may result from subcellular sequestration of MPP⁺ within the norepinephrine neuron's catecholamine storage vesicle. However, these researchers did not isolate or identify a vesicle membrane transport protein responsible for uptake of MPP⁺.

Rat pheochromocytoma 12 (PC12) cells derive from the adrenal gland and, although they have served as a model system to understand MPP⁺ toxicity, PC12 cells show little susceptibility to the toxin, with 1 mM MPP⁺ resulting in cell death by four days and 100 μM MPP⁺ resulting in death by two weeks (Greene and Rein, *Brain Res.* 129:247-263 (1978); and Denton and Howard, *J. Neurochem.* 49:622-630 (1987)). Furthermore, inhibition of high-affinity plasma membrane uptake by tricyclic antidepressant drugs protects PC12 cells entirely from MPP⁺, indicating that without an active system for its accumulation, these cells are intrinsically resistant to the effects of the toxin (Snyder et al., supra). Therefore, the resistance of PC12 cells to MPP⁺ toxicity can be used to dissect the differential MPP⁺ susceptibility of aminergic cell populations in the midbrain, and adrenal gland as well as perhaps sympathetic ganglia.

In addition to MPP⁺, exposure of neurons to other toxic compounds, including the excitatory neurotransmitters glutamate and aspartate, may be implicated in diseases. For example, N-methyl-D-aspartate (NMDA) antagonists prevent hypoxia as well as other forms of neuronal injury (Choi et al., *Annu. Rev. Neurosci.* 13:171-182 (1990)). Effective sequestration of such toxic compounds into vesicles may prevent neuronal injury.

Transport and intracellular sequestration of various amines has implications for the diagnosis and treatment of psychiatric disorders including affective disorders (Carlsson, *J. Psychopharmacol.* 4:12-126 (1990)) and schizophrenia (Wyatt et al., *Schizophr. Res.* 1:3-18 (1988)) in which an imbalance of amines is involved.

Vesicle membrane transport proteins have been identified (Johnson, *Physiol. Rev.* 68:232-307 (1988); Anderson et al., *Biochem.* 21:3037-3043 (1982); Hell et al., *EMBO J.* 7:3023-3029 (1988); Kish et al., supra; Maycox et al., supra; and Carlson et al., supra) but have not been isolated, cloned and expressed. It is difficult to clone mammalian vesicle membrane transport proteins in part because of the difficulties in purifying low abundance, hydrophobic membrane proteins that constitute less than 0.2% of the membrane protein. Thus, these proteins are available in small quantities only from mammalian vesicle membranes. Therefore, it would be desirable to have available a method for producing practical quantities of vesicle membrane transport proteins and for studying the properties and uses of these proteins.

It would be particularly desirable to determine the location of the chromosomal gene for human central nervous system synaptic vesicle amine transporter as well as to produce a cDNA coding for this gene product, in order to produce useful quantities of this protein for studies to determine the relationship of biogenic amine metabolism to human disease.

A wide range of clinical disorders and pharmacologic agents have implicated the monoamines in consciousness, motivation, the organization of thought, mood, motor control, sensory perception and such autonomic phenomena as heart rate, vascular tone, and blood pressure. However, the role of monoamines in human disease remains unclear.

In particular, it is not known whether changes in monoamine metabolism cause the various disorders or results from

them. Nonetheless, diverse human conditions including affective disease, schizophrenia, and vascular headache have a substantial inherited component, suggesting that a genetic defect in synaptic transmission by the monoamines may be responsible. However, the difficulty in diagnosis of the syndromes and their probable genetic heterogeneity complicate attempts to identify the genes responsible by standard linkage analysis (K. K. Kidd, *Social Biol.* 38:163-178 (1991)).

On the other hand, components of the signaling apparatus from monoamines indicate specific candidate genes. The dramatic behavioral effects of drugs that affect monoamine transport further suggest the corresponding genes as candidates of particular importance for human neuropsychiatric illness (G. F. Koob & F. E. Bloom, *Science* 242:715-723 (1988)).

Although the molecular basis for many aspects of synaptic transmission by the monoamines has been characterized in considerable detail, the role of transport in this process has eluded characterization at the molecular level until relatively recently. Two types of transport activity participate in synaptic transmission by the amines (B. I. Kanner & S. Schuldiner, *CRC Crit. Rev. Biochem.* 22:138 (1987)). One type occurs at the plasma membrane and terminates the action of the neurotransmitter by removing it from the synapse into the cytoplasm of the presynaptic cell. This transport activity uses the cotransport of Na⁺ to drive uptake of the monoamine. Cocaine and the tricyclic antidepressants act by inhibiting this activity, which presumably increases the synaptic level of neurotransmitter and so elevates mood. (J. Axelrod et al., *Science* 133:383-384 (1961); L. L. Iversen, "The Uptake of Biogenic Amines" in *Handbook of Psychopharmacology* (S. D. Iversen & S. H. Snyder, eds., Plenum Press, New York (1976)).

Molecular cloning has identified three distinct plasma membrane monoamine transporters, one for norepinephrine (T. Pacholczyk et al., *Nature* 350:350-354 (1991)), another for dopamine (B. Giros et al., *FEBS Lett.* 295:149-154 (1991); J. E. Kilty et al., *Science* 254:578-579 (1991); S. Shimada et al., *Science* 254:576-578 (1991); T. B. Usdin et al., *Proc. Natl. Acad. Sci. USA* 88:1168-1171 (1991)), and a third for serotonin (R. D. Blakeley et al., *Nature* 354:66-70 (1991); B. J. Hoffman et al., *Science* 254:579-580 (1991)). In addition, the similarity of these transporters to the plasma membrane transporters for a variety of other classical transmitters indicates that these proteins comprise a large, closely related family (G. R. Uhl, *Trends Neurosci.* 15:265-268 (1992)). The plasma membrane glutamate transporters form yet another distinct family (Y. Kanai & M. A. Hediger, *Nature* 360:467-471 (1992); G. Pines et al., *Nature* 360:464-467 (1992); T. Storck et al., *Proc. Natl. Acad. Sci. USA* 89:10955-10959 (1992)).

In contrast, the vesicular amine transporters serve a different function in synaptic transmission, have a distinct mechanism and pharmacology, and show no structural similarity to the large group of characterized plasma membrane transporters. Vesicular monoamine transport activity packages neurotransmitter so that its release can be regulated by neuroactivity (P. D. de Camilli & R. Jahn, *Ann. Rev. Physiol.* 52:625-645 (1990); R. B. Kelly, *Curt. Opin. Cell Biol.* 3:654-660 (1991); W. S. Trimble et al., *Ann. Rev. Neurosci.* 14:93-122 (1991)). The activity occurs on the membrane of vesicles rather than on the plasma membrane and functions as a proton exchanger, using the proton electrochemical gradient generated by the vesicular H⁺-ATPase to drive the uptake of monoamines from the cytoplasm. Reserpine and tetrabenazine inhibit this transport activity, and in contrast to

the inhibitors of plasma membrane amine transport, may induce rather than alleviate a form of depression (E. D. Frize, *New Engl. J. Med.* 251:1006-1008 (1954)). Thus, vesicular amine transport plays a critical role in synaptic transmission by the monoamines and a defect in its expression or regulation may contribute to psychiatric disease.

Because of this likely role of vesicular amine transport proteins in both Parkinson's Disease and psychiatric disease states, it would therefore be highly desirable to isolate both genomic DNA for the human central nervous system membrane vesicle amine transport protein, as well as cDNA corresponding to the messenger RNA for the protein. Such isolated genomic DNA and cDNA would allow the determination of the similarity of the sequences involved to those of the chromaffin granule amine transporter, and would allow the use of the nucleic acid as a probe for diagnostic purposes, for expression in suitable host cells to produce recoverable quantities of the protein, and for genetic therapy of disease. Such recoverable quantities of the protein could be used for studies of drugs and toxins for their effect on the conditions believed to be associated with central nervous system vesicle monoamine transport activity.

SUMMARY OF THE INVENTION

The invention provides a means for obtaining mammalian vesicle membrane transport proteins in quantity.

Thus, in one aspect, the invention relates to recombinantly produced mammalian vesicle membrane transport protein. This protein has an amino acid sequence substantially similar to that shown in FIG. 1, (SEQ ID NO: 2) FIG. 2 (SEQ ID NO: 4) or FIG. 3 (SEQ ID NO: 13). FIG. 1 shows the nucleotide and amino acid sequence of a rat chromaffin granule amine transport protein (rCGAT), FIG. 2 the nucleotide and amino acid sequence of a rat synaptic vesicle amine transport protein (rSVAT), and FIG. 3 the nucleotide and amino acid sequence of a human synaptic vesicle amine transport protein (hSVAT) (SEQ ID NO: 1, 2, 3, 4, 12, & 13). Functional equivalents of transport protein having either the amino acid sequence of FIG. 1, FIG. 2, or FIG. 3 (SEQ ID NO: 2, 4, & 13) and having vesicle membrane transport activity are also within the invention. In particular, the proteins of the invention include proteins related to the proteins depicted in FIG. 1, FIG. 2, or FIG. 3 (SEQ ID NO: 2, 4, & 13) by one or more conservative amino acid substitutions substantially preserving the structure of the transmembrane domains.

Also within the scope of the invention is a protein having vesicular amine transport activity and encoded by genomic DNA having at least about 60% homology in its exons with at least one of the DNA sequences of FIG. 1, FIG. 2, or FIG. 3 (SEQ ID NO: 1, 3, & 12).

The invention further relates to cDNA sequences and genomic DNA sequences encoding mammalian vesicle membrane transport protein, to expression vectors suitable for production of this protein, to recombinant host cells transformed with these vectors, and to methods for producing recombinant vesicle membrane transport protein.

Among the genomic DNA sequences within the scope of the invention is substantially purified genomic DNA whose exons encode mammalian vesicle membrane transport protein.

Among the genomic DNA sequences within the scope of the invention is a human genomic DNA sequence, particularly a human genomic DNA sequence having at least about 70% homology in one of its exons with a portion of the cDNA sequence shown in FIG. 2 extending from about base 220 to about base 540 (SEQ ID NO: 3).

Another aspect of the invention is an expression vector for mammalian vesicle membrane transport protein comprising a cDNA sequence according to the present invention operably linked to at least one control sequence compatible with a suitable host cell.

Yet another aspect of the invention is a host cell transformed with an expression vector according to the present invention in a manner allowing the transformed host cell to express the transport protein encoded by the cDNA incorporated within the expression vector in a detectable quantity.

Another aspect of the invention is a method for obtaining at least one gene that encodes mammalian vesicle membrane transport protein. The method comprises hybridizing a cDNA according to the present invention as a detectable probe with DNA containing at least one gene encoding mammalian vesicle membrane transport protein in order to obtain the gene in substantially purified form.

Another aspect of the invention is a method for producing adrenal-specific or brain-specific mammalian vesicle membrane transport protein comprising:

- (1) culturing a host cell according to the present invention transformed with an appropriate expression vector;
- (2) using the cultured host cell to express the transport protein; and
- (3) purifying the protein.

In other aspects, the invention relates to compositions containing mammalian vesicle membrane transport protein, and to methods of using these compositions. These methods include a method for screening a cytotoxic reagent to determine whether cytotoxicity of the reagent is reduced by sequestration of the reagent in intracellular vesicles. The methods of the invention also include a method for identifying a compound that selectively inhibits or activates the activity of a membrane transport protein, and a method for identifying a tissue-specific inhibitor of the transport protein.

In another aspect, the invention relates to methods for the qualitative and quantitative assay of vesicular membrane transport protein.

In yet another aspect, the invention relates to antibodies specific for transport protein and to methods of using these antibodies. The antibodies can be polyclonal or monoclonal.

In yet another aspect, the invention relates to a method for detecting a chromosomal abnormality in human chromosome 10. The method comprises:

- (1) hybridizing a labeled single-stranded nucleic acid sequence of sufficient length to hybridize specifically to chromosomal DNA and whose sequence is derived from the human SVAT gene to human chromosomal DNA; and
- (2) detecting the hybridized labeled nucleic acid sequence to determine the presence or absence of an abnormality in human chromosome 10.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features, aspects, and advantages of the present invention will become better understood with reference to the following description, appended claims, and accompanying drawings where:

FIG. 1 is the cDNA sequence and predicted amino acid sequence of the rat chromaffin granule amine transporter protein, as described in Examples 1 and 2, *infra* (nucleotide sequence of cDNA clone mpp^{res}, with the deduced amino acid sequence below; transmembrane domains are underlined and potential sites for N-linked glycosylation are indicated by an asterisk below) (SEQ ID NO: 1 and 2).

FIG. 2 is the cDNA sequence and predicted amino acid sequence of the rat synaptic vesicle amine transporter protein, as described in Example 3, *infra*, with transmembrane domains and potential sites for N-linked glycosylation indicated as in FIG. 1 (SEQ ID NO: 3 and 4).

FIG. 3 is the cDNA sequence and predicted amino acid sequence of the human synaptic vesicle amine transporter protein, as described in Example 4, *infra*, with transmembrane domains and potential sites for N-linked glycosylation indicated as in FIG. 1 (SEQ ID NO: 12 and 13).

FIG. 4 is a drawing showing the homology between the rat chromaffin granule amine transporter protein and the rat synaptic vesicle amine transporter protein, with identical residues in black and conservative changes in shading (SEQ ID NO: 2 and 4).

FIG. 5 is the sequence (SEQ ID NO: 5) of a portion of human genomic DNA hybridizing to the cDNA sequence of FIG. 2 (SEQ ID NO: 3), showing the homology.

FIG. 6a and 6b are graphs showing the differential susceptibility to the respiratory complex I inhibitors MPP⁺ and rotenone, in wild-type and MPP⁺-resistant CHO cells as described in Example 1, *infra* (FIG. 6a: MPP⁺; FIG. 6b: rotenone).

FIG. 7a and 7b are graphs showing the effects on oxygen consumption of MPP⁺ in wild-type (FIG. 7a) and MPP⁺-resistant (FIG. 7b) cells, as described in Example 1, *infra*.

FIG. 8a and 8b are graphs showing the effects on MPP⁺ resistance of reserpine as described in Example 1, *infra* (FIG. 8a: MPP⁺-resistant CHO cells at 25% confluence; FIG. 8b: wild-type CHO cells).

FIG. 9a-9c are photographs showing particulate staining of exogenously loaded dopamine in a MPP⁺-resistant CHO transformant using glyoxylic acid-induced fluorescence, as described in Example 1, *infra* (FIG. 9a: wild-type CHO cells; FIG. 9b and 9c: MPP⁺-resistant CHO cells).

FIG. 10 is a diagrammatic depiction from above of presynaptic neurons with mitochondrion (rectangle), synaptic vesicles, equal plasma membrane uptake (large arrows) but less (left) and more (right) vesicular uptake (small arrows), as described in Example 1, *infra*. (The concentration of toxin or neurotransmitter in the various compartments is indicated by the intensity of shading).

FIG. 11 is a photograph showing expression of RNA transcripts from the rescued *mpp^{res}* sequences in PC12 cells, wild-type and MPP⁺-resistant CHO cells, as described in Example 2, *infra*.

FIG. 12a-12c are photographs of glyoxylic acid-induced fluorescence of cells loaded with exogenous dopamine as described in Example 2, *infra* (FIG. 12a: wild-type CHO cells; FIG. 12b: MPP⁺-resistant CHO cells; FIG. 12c: effects of reserpine on MPP⁺-resistant CHO cells).

FIG. 13 is a photograph showing selection of CHO cells in MPP⁺ after transfection with cDNA clone *mpp^{res}* (left) and CDM8 vector alone (right), as described in Example 2, *infra*.

FIG. 14 is a photograph showing glyoxylic acid-induced fluorescence of *mpp^{res}* transformants selected in the neomycin analogue G418 in the absence (top) and presence (bottom) of reserpine, as described in Example 2, *infra*.

FIGS. 15A-15C are graphs showing the results of a quantitative determination of dopamine uptake by *mpp^{res}* cells (FIG. 15A: kinetics of incorporation; FIG. 15B: determination of K_m for dopamine by Lineweaver-Burke plot; FIG. 15C: dose-response study of inhibition by reserpine, tetrabenazine, and cocaine).

FIG. 16 is a model of the predicted structure of the chromaffin granule amine transport protein as described in Example 2, *infra* (lumen of the granule is above, cytoplasm below; basic and acidic residues are shown as "+" and "-", respectively, with N-linked carbohydrates in the first luminal loop indicated by a branched structure).

FIG. 17 is a diagram showing the alignment of the N-terminal domains of the methylenomycin^{res} (Mmr) (SEQ ID NO: 6), tetracycline^{res} pBR (SEQ ID NO: 7), Tet Tn10 (SEQ ID NO: 8) and bacterial multi-drug resistance (BMR) (SEQ ID NO: 9) transporters, with the vesicle membrane amine transport protein (CGAT) (SEQ ID NO: 10) (part of SEQ ID NO: 2) as described in Example 2, *infra* (shared residues are capitalized and shown below as a consensus sequence (SEQ ID NO: 11); the regions in brackets indicate the predicted transmembrane helices).

FIG. 18 is a gel showing the results of Northern blotting of mRNA from various tissues showing the differential expression of the chromaffin granule and synaptic vesicle transport proteins (FIG. 18A: chromaffin granule transport protein cDNA of FIG. 1 as probe; FIG. 18B: synaptic vesicle transport protein cDNA of FIG. 2 as probe).

FIG. 19 is an autoradiograph showing the results of *in situ* hybridization of an antisense RNA probe corresponding to the synaptic vesicle amine transport protein to sections of the brain. (FIG. 19A: substantia nigra (SN) and ventral tegmental area (VTA); FIG. 19B: locus coeruleus (LC) and region A5 (A5); FIG. 19C: nucleus raphe pallidus (nrp), nucleus tractus solitarius (nts), and region A1 (A1)).

FIG. 20 is an autoradiograph showing the results of polymerase chain reaction (PCR) analysis using two sets of primers (SEQ ID NO: 14, 15, 16, and 17) in order to localize the human SVAT gene to chromosome 10. (IMR: human genomic DNA; GM: mouse genomic DNA).

FIG. 21 is a diagram showing a comparison of the amino acid sequences (SEQ ID NO: 4, 2, and 13) predicted to result from translation of the cDNA sequences for rat SVAT, rat CGAT, and human SVAT (SEQ ID NO: 3, 1, and 12), as aligned for maximum homology.

FIG. 22 is a diagram showing a comparison of the cDNA sequences for rat CGAT and human SVAT (SEQ ID NO: 1 and 12), as aligned for maximum homology, and for rat SVAT and human CGAT (SEQ ID NO: 3 and 12), as aligned for maximum homology.

FIG. 23 is an autoradiograph showing the results of Southern blot analysis localizing the human CGAT gene to chromosome 8, with mouse (GM) and human (IMR) DNA.

FIG. 24 is a diagram based on autoradiographic data showing the results of *in situ* hybridization localizing the human CGAT gene to chromosome 8p, indicated by the distribution of silver grains.

FIG. 25 is a photograph showing the results of fluorescent *in situ* hybridization localizing the human SVAT gene to chromosome 10q, the single positively hybridizing chromosome indicated by the arrow.

FIG. 26 is a diagram showing the results of sequential T-G banding to demonstrate localization of grain counts for *in situ* hybridization to chromosome 8p21.3 for human CGAT, and chromosome 10q25 for human CGAT.

DESCRIPTION

In order that the invention herein described may be more fully understood, the following description is set forth.

Definitions

As used herein, "mammalian vesicle membrane transport protein" refers to the mammalian vesicle membrane trans-

port protein expressed from a clone obtained as described below. This protein can be either of rat or human origin. Proteins of rat origin include adrenal-specific chromaffin-granule amine transporter protein (rCGAT protein) and brain-specific synaptic vesicle amine transporter protein (rSVAT protein). Proteins of human origin include brain-specific synaptic vesicle amine transporter protein (hSVAT protein). These proteins have an amino acid sequence substantially similar to the amino acid sequences shown in FIGS. 1, 2, or 3, (SEQ ID NO: 2, 4, & 13) but minor modifications of this sequence which do not substantially alter activity also fall within the definition and within the protein of the invention. Also included within the definition are fragments of the entire sequence encoding the protein which retain activity. This protein has no homology with the amino acid sequence of known proteins from other mammalian tissues and remote homology with a class of bacterial transport proteins.

As is the case for all proteins, vesicle membrane transport protein can occur in neutral form or in the form of basic or acid addition salts, depending on its mode of preparation, or, if in solution, upon its environment. In addition, the protein can be modified by combination with other biological materials such as lipids and carbohydrates, or by side chain modification, such as acetylation of amino groups, phosphorylation of hydroxyl side chains, or oxidation of sulfhydryl groups, or other modification of the encoded primary sequence. In its native form, the vesicle membrane transport protein is probably a glycosylated protein and is associated with phospholipids. Included within the definition of the protein herein are glycosylated and unglycosylated forms, hydroxylated and nonhydroxylated forms, and any composition of an amino acid sequence substantially similar to that shown in FIGS. 1 or 2 (SEQ ID NO: 2 & 4) which retains the ability of the protein to transport substances including amine transmitters as well as acetylcholine, glutamate, glycine and gamma-aminobutyric acid (GABA), across vesicle membranes.

It is further understood that minor modifications of primary amino acid sequence may result in proteins that have substantially equivalent or enhanced activity as compared to the sequences set forth in FIG. 1, FIG. 2 or FIG. 3 (SEQ ID NO: 1, 2, 3, 4, 12 & 13). These modifications can be deliberate, as by site-directed mutagenesis, or can be accidental, for example by mutation of hosts that are organisms that produce the vesicle membrane transport protein. All of these modifications are included in the definition provided that activity of the protein is retained.

The term "functional equivalents" as used herein refers to proteins having physical and chemical properties substantially equivalent to proteins whose primary amino acid sequences are set forth in FIG. 1, FIG. 2, or FIG. 3 (SEQ ID NO: 2, 4, & 13). These properties include molecular weight, state of aggregation, charge, transport activity, and affinity for substrates, inhibitors, and/or modulators of activity.

"Activity" of the protein is defined as transport into a vesicle derived from transfected cells or into liposomes reconstituted with the transport protein. For example, neural or non-neural mammalian cells, or cells from lower organisms are transfected with the cloned cDNA encoding the vesicle membrane transport protein in a suitable expression vector. The membranes are then isolated from the transfected cells after homogenization, and are incubated, e.g. at 20°–37° C. for from 2 to 45 minutes, in an appropriate buffer containing ATP. The uptake of radiolabelled neurotransmitters is then measured by filtering the mixture and counting the bound fluid material using a scintillation counter. In

addition, the vesicle membrane transport protein can be reconstituted in lipid vesicles and uptake into these vesicles is measured as above in the presence of a superimposed electrochemical gradient needed to drive transport developed in vivo by the vesicular ATPase, or in vitro by either the endogenous ATPase, an exogenous ATPase or an artificial gradient. Carlson et al., *J. Biol. Chem.* 264:7369–7376 (1989); Maycox et al., *J. Biol. Chem.* 263:15423–15428 (1988); Maycox et al., *EMBO J* 9:1465–1469 (1990); Hell et al., *J. Biol. Chem.* 265:2111–2117 (1990) and Hell et al., *Biochem.* 30:11795–11800 (1991).

"Control sequence" refers to a DNA sequence or sequences which are capable, when properly ligated to a desired coding sequence, of effecting its expression in hosts compatible with such sequences. Such control sequences include promoters in both procaryotic and eucaryotic hosts, and in procaryotic organisms also include ribosome binding site sequences, and, in eucaryotes, termination signals. Additional factors necessary or helpful in effecting expression may subsequently be identified. As used herein, "control sequences" simply refers to whatever DNA sequence may be required to effect expression in the particular host employed.

"Operably linked" refers to a positional arrangement wherein the components are configured so as to perform their usual function. Thus, control sequences operably linked to coding sequences are capable of effecting the expression of the coding sequence.

"Cells" or "recombinant host cells" or "host cells" are often used interchangeably herein as will be clear from the context. These terms include the immediate subject cell, and the progeny thereof. It is understood that not all progeny are exactly identical to the parental cell, due to chance mutations or differences in environment. However, such altered progeny are included when the above terms are used.

I. METHODS FOR OBTAINING AND EXPRESSING cDNA CODING FOR MAMMALIAN VESICLE MEMBRANE TRANSPORT PROTEIN

The methods illustrated below to obtain a cDNA sequence encoding mammalian vesicle membrane transport protein, the gene for the transport protein and the transport protein, are merely for purposes of illustration and are typical of those that might be used. However, other procedures may also be employed, as is understood in the art.

Chinese hamster ovary (CHO) cells lack several neuronal features known to affect susceptibility to MPP⁺, such as excitability, catecholamine uptake activity and receptors for neurotrophic factors. However, at high density, CHO cells have an extremely steep dose-response curve to MPP⁺, with virtually complete inhibition of protein synthesis after exposure to over 100 μM for only 2–3 days. At low density, CHO cells show a similar threshold of sensitivity, but simply stop growing until detachment from the plate days to weeks later. Although they lack a plasma membrane catecholamine transport protein and so have less sensitivity to MPP⁺ than dopaminergic neurons in vivo, CHO cells still show greater sensitivity to MPP⁺ than PC12 cells. Thus, CHO cells were first used herein as a simplified system in which to identify the properties that confer resistance to this form of toxicity in PC12 cells.

Cloning of Coding Sequences for Mammalian Vesicle Membrane Transport Protein

The entire cDNA sequence encoding rat chromaffin granule transport protein has been cloned and expressed in Chinese Hamster Ovary (CHO) cells as set forth in Examples 1 and 2, *infra*.

A PC12 cDNA library in a plasmid expression vector was transferred into relatively resistant MPP⁺-sensitive CHO

fibroblasts using a modified calcium phosphate transfection procedure as described by Chen and Okayama, *Mol. Cell. Biol.* 7:2745-2752 (1987)). Selection of the stable transformants in 1 mM MPP⁺ yielded a clone extremely resistant to MPP⁺. Plasmids integrated into the host cell DNA were rescued to identify the cDNA sequences responsible for conferring resistance to MPP⁺. Retransfection of the pooled rescued DNA produced secondary transformants exhibiting resistance to MPP⁺. This resistance was found to be reversible with reserpine.

The entire cDNA sequence encoding human brain-specific synaptic vesicle amine transporter protein (hSVAT) has also been cloned as set forth in Example 4, *infra*. A human midbrain cDNA library in λ bacteriophage gt10 was screened with rat rSVAT cDNA, positive plaques were picked, and bacteriophage DNA prepared from the plaques. To identify clones containing full-length cDNA, oligonucleotide primers from the 5'- and 3'- ends of rSVAT were used to amplify the bacteriophage DNA by the polymerase chain reaction (PCR) procedure. A single clone yielded a PCR product of the appropriate size, and the insert from this clone was subcloned for sequence analysis.

Genomic DNA in a λ bacteriophage vector library was also used to isolate substantially purified DNA comprising the gene for hSVAT, including the introns originally present in the genome. The genomic library was screened with radioactively labeled rCGAT cDNA. Positive plaques were picked after autoradiography and purified through additional rounds of screening. To distinguish between phage clones encoding CGAT and SVAT, a radiolabeled Nco I fragment containing a divergent loop between the first and second predicted transmembrane domains was used to probe a Southern blot of DNA from the various phage clones. A 1.3 kb Eco RI fragment from one phage isolate was subcloned. Sequence analysis of double-stranded plasmid DNA of this subclone showed an exon with a predicted amino acid sequence highly related to a large loop in rSVAT and unrelated to the same region of rCGAT.

Expression of Mammalian Vesicle Membrane Transport Protein

With the complete nucleotide sequence encoding mammalian vesicle membrane transport protein provided herein, the sequence can be expressed in a variety of systems. In Example 1, *infra*, the cDNA encoding the protein is expressed in CHO cells. To effect functional expression, the plasmid expression vector CDM8 (Aruffo and Seed, *Proc. Natl. Acad. Sci. USA* 84:8573-8577 (1987), provided by Drs. Aruffo and Seed (Harvard University, Boston, Mass.) was used. Alternatively, other suitable expression vectors such as retroviral vectors can be used. The vector containing the cDNA was then transfected into CHO cells, and stable transformants were selected in MPP⁺. Expression of the vesicle membrane transport protein in CHO cells resulted in cells resistant to the toxin MPP⁺ whose resistance was reversed in the presence of reserpine. In addition, cells resistant to the toxin showed a particulate cytoplasmic stain for loaded dopamine that was also reversed by reserpine.

Standard Methods

The techniques for sequencing, cloning and expressing DNA sequences encoding the amino acid sequences corresponding to the vesicle membrane transport protein, e.g. polymerase chain reaction (PCR), synthesis of oligonucleotides, probing a cDNA library, transforming cells, constructing vectors, extracting messenger RNA, preparing cDNA libraries, and the like are well-established in the art, and most practitioners are familiar with the standard resource materials for specific conditions and procedures.

However, the following paragraphs are provided for convenience and notation of modifications where necessary, and may serve as a guideline.

Sequencing

Isolated cDNA encoding the vesicle membrane transport protein was analyzed by subcloning the insert from the cDNA clone into a plasmid such as pBluescriptTM (Stratagene, San Diego, Calif.) and using the dideoxy method (Sanger et al., *Proc. Natl. Acad. Sci. USA* 74:5463-5467 (1977)) using single-stranded templates and Sequenase (U.S. Biochemical (USB), Cleveland, Ohio). The DNA sequence and predicted protein sequence was then compared to established databanks such as Gen Bank, EMBO or SwissProt using standard as well as profile-based methods as described by Gribskov et al., *Proc. Natl. Acad. Sci. USA* 84:4355-4358 (1987) and Devereux et al., *Nucl. Acids Res.* 12:387-395 (1984).

Hosts and Control Sequences

Both procaryotic and eucaryotic systems can be used to express the vesicle membrane transport protein; however eucaryotic hosts are preferred.

Eucaryotic microbes, such as yeast, can be used as hosts for mass production of the vesicle membrane transport protein. Laboratory strains of *Saccharomyces cerevisiae*, Baker's yeast, are most used although a number of other strains are commonly available. Vectors employing, for example, the 2 μ origin of replication of Broach, *Meth. Enz.* 101:307 (1983), or other yeast compatible origins of replications (see, for example, Stinchcomb et al., *Nature* 282:39 (1979)); Tschempe et al., *Gene* 10:157 (1980); and Clarke et al., *Meth. Enz.* 101:300 (1983)) can be used.

Control sequences for yeast vectors include promoters for the synthesis of glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg.* 7:149 (1968); Holland et al., *Biochemistry* 17:4900 (1978)). Additional promoters known in the art include the promoter for 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.* 255:2073 (1980)), and those for other glycolytic enzymes.

Other promoters, which have the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytocrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, and enzymes responsible for maltose and galactose utilization.

It is also believed terminator sequences are desirable at the 3' end of the coding sequences. Such terminators are found in the 3' untranslated region following the coding sequences in yeast-derived genes.

Alternatively, the genes encoding the mammalian vesicle membrane transport protein are expressed in eucaryotic host cell cultures derived from multicellular organisms. (See, for example, *Tissue Cultures*, Academic Press, Cruz and Patterson, Eds. (1973)). These systems have the additional advantage of the ability to splice out introns and thus can be used directly to express genomic fragments.

Useful host cell lines include amphibian oocytes such as *Xenopus* oocytes, COS cells, VERO and HeLa cells, Chinese hamster ovary (CHO) cells and insect cells such as SF9 cells. Expression vectors for such cells ordinarily include promoters and control sequences compatible with mammalian cells such as, for example, the commonly used early and late promoters from baculovirus, vaccinia virus, Simian Virus 40 (SV40) (Fiers et al., *Nature* 273:113 (1973)), or other viral promoters such as those derived from polyoma, Adenovirus 2, bovine papilloma virus, or avian sarcoma viruses. The controllable promoter, hMTII (Karin et al., *Nature* 299:797-802 (1982)) may also be used.

General aspects of mammalian cell host system transformations have been described by Axel (U.S. Pat. No. 4,399, 216 issued Aug. 16, 1983). It now appears, that "enhancer" regions are important in optimizing expression; these are, generally, sequences found upstream or downstream of the promoter region in non-coding DNA regions. Origins of replication can be obtained, if needed, from viral sources. However, integration into the chromosome is a common mechanism for DNA replication in eucaryotes.

If procaryotic systems are used, an intronless coding sequence should be used, along with suitable control sequences. The cDNA of mammalian vesicle membrane transport protein can be excised using suitable restriction enzymes and ligated into procaryotic vectors along with suitable control sequences for such expression.

Procaryotes most frequently are represented by various strains of *E. coli*; however, other microbial strains may also be used. Commonly used procaryotic control sequences which are defined herein to include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequences, include such commonly used promoters as the beta-lactamase (penicillinase) and lactose (*lac*) promoter systems (Chang et al., *Nature* 198:1056 (1977)) and the tryptophan (*trp*) promoter system (Goeddel et al., *Nucleic Acids Res.* 8:4057 (1980)) and the lambda derived P_L promoter and N-gene ribosome binding site (Shimatake et al., *Nature* 292:128 (1981)).

Transformations

Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The treatment employing calcium chloride, as described by Cohen, *Proc. Natl. Acad. Sci. USA* (1972) 69:2110 (1972) or the $CaCl_2$ method described in Maniatis, et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Sambrook et al., 2nd edition, (1989)) can be used for procaryotes or other cells which contain substantial cell wall barriers. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, *Virology* 52:546 (1978), optionally as modified by Wigler et al., *Cell* 16:777-785 (1979), or by Chen and Okayama, *supra*, can be used. Transformations into yeast can be carried out according to the method of Van Solingen et al., *J. Bact.* 130:946 (1977), or of Hsiao et al., *Proc. Natl. Acad. Sci. USA* 76:3829 (1979).

Other representative transfection methods include viral transfection, DEAE-dextran mediated transfection techniques, lysozyme fusion or erythrocyte fusion, scraping, direct uptake, osmotic or sucrose shock, direct microinjection, indirect microinjection such as via erythrocyte-mediated techniques, and/or by subjecting host cells to electric currents. The above list of transfection techniques is not considered to be exhaustive, as other procedures for introducing genetic information into cells will no doubt be developed.

Cloning

The cDNA sequences encoding the rSVAT and rCGAT protein were obtained from an oligo-dT-primed PC12 cDNA library.

Alternatively, the cDNA sequences encoding mammalian vesicle membrane transport protein are obtained from a cDNA library prepared from mRNA isolated from cells expressing the vesicle membrane protein in various organs such as the brain, according to procedures described in *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, second edition, Sambrook et al., eds., (1989). The cDNA insert from the successful clone, excised with a restriction enzyme such as Eco RI, is then used as a probe

of the original cDNA library to obtain the additional clones containing inserts encoding other regions of the vesicle membrane protein, that, together with this probe, span the nucleotides containing the complete protein coding sequence of the protein.

An additional procedure for obtaining cDNA sequences encoding the vesicle membrane transport protein is PCR. PCR is used to amplify sequences from a pooled cDNA library of reverse-transcribed RNA, using oligonucleotide primers based on the transporter sequences already known.

Double-stranded cDNA encoding vesicle membrane transport protein is synthesized and prepared for insertion into a plasmid vector such as Bluescript® or Lambda ZAP® (Stratagene, San Diego, Calif.) or a vector from Clontech, Palo Alto, Calif., using standard procedures (see *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, Sambrook et al., eds. second edition (1989)).

Library Screening

The selected cDNA library is screened using reduced stringency conditions as described by Ausubel et al., in *Current Protocols in Molecular Biology*, Greene Publishing and Wiley-Interscience, New York (1990) or using methods described in *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, Sambrook et al., eds., second edition (1989), or using a plaque hybridization procedure with a fragment of the cDNA coding for rat adrenal vesicle membrane transport protein.

Plaque hybridization is typically carried out as follows. Host bacteria such as *E. coli* C600 Hfl or LE 392 (Stratagene) are grown overnight at 30° C. in 1% casein hydrolysate, 0.5% NaCl, 0.5% yeast extract, 0.1% casamino acids, 0.2% $MgSO_4$ (NZYCM) medium (*Molecular Cloning: A Laboratory Manual*, *supra*), gently pelleted and resuspended in one half the original volume of 10 mM $MgSO_4$. After titration, an amount of the phage library containing approximately 50,000 plaque forming units (pfu) is added to 300 μ l of the host bacteria, incubated at 37° C. for 15 minutes and plated onto NZYCM agar with 10 ml NZYCM top agarose. A total of a million plaques distributed on 20 fifteen cm plates are screened. After the plaques have grown to 1 mm, the plates are chilled at 4° C. for at least two hours, and then overlaid with duplicate nylon filters, followed by denaturation of the filters in 0.5M NaOH/1.5M NaCl for five minutes and neutralization in 0.5M Tris, pH 7.4/1.5M NaCl for five minutes. The filters are then dried in air, baked at 80° C. for two hours, washed in 5 \times SSC/0.5% SDS at 68° C. for several hours, and prehybridized in 0.5M $NaPO_4$, pH 7.2/1% BSA/1 mM EDTA/7% SDS/100 μ g/ml denatured salmon sperm DNA for more than 4 hours.

Using the rat chromaffin granule amine transport cDNA described herein labeled by random priming as a probe, high stringency hybridization is carried out in the same solution at 68° C., and the temperature is reduced to 50°-60° C. for lower stringency hybridization. After hybridization for 16-24 hours, the filters are washed first in 40 mM $NaPO_4$, pH 7.2/0.5% BSA/5% SDS/1 mM EDTA twice for one hour each, then in 40 mM $NaPO_4$, pH 7.2/1% BSA/1 mM EDTA for one hour each, both at the same temperature as the hybridization (Boulton et al., *Cell* 65:663-675 (1991)). The filters will then be exposed to film with an enhancing screen at -70° C. for one day to one week.

Positive signals are then aligned to the plates, and the corresponding positive phage is purified in subsequent rounds of screening, using the same conditions as in the primary screen. Purified phage clones are then used to prepare phage DNA for subcloning into a plasmid vector for

sequence analysis. The various independent clones are also analyzed in terms of tissue distribution, using Northern blots and in situ hybridization using standard methods, as well as in terms of function, using expression in a heterologous eucaryotic expression system such as CHO cells.

In order to isolate the vesicle membrane transport cDNA from mammalian brain cells, for example rat brain, a random-primed, rat brainstem cDNA library in lambda gt10 (Clontech) was screened using the rat cDNA encoding the rat membrane transport protein described herein. In order to obtain the transport cDNA from human cells, a human cDNA library was also screened using a rat cDNA probe.

RNA Preparation

RNA preparation is as follows. The samples used for preparation of RNA are immediately frozen in liquid nitrogen and then stored until use, up to one week at -80°C . The RNA is prepared by CsCl centrifugation (Ausubel et al., supra, incorporated by reference herein) using a modified homogenization buffer (Chirgwin et al., *Biochem.* 18:5294-5299 (1979)). Poly(A⁺)RNA is selected by oligo (dT) chromatography (Aviv and Leder, *Proc. Natl. Acad. Sci. USA* 69:1408-1412 (1972)). RNA samples are stored at -80°C .

Northern Blots

Analysis of gene expression and tissue distribution can be accomplished using Northern blots containing mRNA from a range of rat tissues and cDNA encoding the rat adrenal chromaffin granule transport protein as described, for example, in Sambrook et al., supra, vol. 1, pp. 7.37-7.52, using radiolabeled cDNA. In this procedure, the mRNA is typically transferred to a nylon membrane or to nitrocellulose. The hybridized radiolabeled cDNA is typically detected using autoradiography.

Vector Construction

Construction of suitable vectors containing the desired coding and control sequences employs standard ligation and restriction techniques which are well understood in the art (Young et al., *Nature* 316:450-452 (1988)).

Site specific DNA cleavage is performed by treating with the suitable restriction enzyme, such as Eco RI, (or enzymes) under conditions which are generally understood in the art, and the particulars of which are specified by the manufacturer of these commercially available restriction enzymes. See, e.g., New England Biolabs, Product Catalog. In general, about 1 μg of phage DNA sequence is cleaved by one unit of enzyme in about 20 μl of buffer solution; in the examples herein, typically, an excess of restriction enzyme is used to ensure complete digestion of the DNA substrate. Incubation times of about one hour to two hours at about 37°C . are workable, although variations can be tolerated. After each incubation, protein is removed by extraction with phenol/chloroform, and can be followed by ether extraction and the nucleic acid recovered from aqueous fractions by precipitation with ethanol.

In vector construction employing "vector fragments", the vector fragment is commonly treated with bacterial alkaline phosphatase (BAP) or calf intestinal alkaline phosphatase (CIP) in order to remove the 5' phosphate and prevent religation of the vector. Digestions are conducted at pH 8 in approximately 150 mM Tris, in the presence of Na⁺ and Mg⁺⁺ using about 1 unit of BAP or CIP per μg of vector at 60°C . or 37°C ., respectively, for about one hour. In order to recover the nucleic acid fragments, the preparation is extracted with phenol/chloroform and ethanol precipitated. Alternatively, religation can be prevented in vectors which have been double digested by additional restriction enzyme digestion of the unwanted fragments.

Ligations are performed in 15-50 μl volumes under the following standard conditions and temperatures: 20 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 10 mM DTT, 33 $\mu\text{g}/\text{ml}$ BSA, 10 mM to 50 mM NaCl, and either 40 μM ATP, 0.01-0.02 (Weiss) units T4 DNA ligase at 0°C . (for "sticky end" ligation) or 1 mM ATP, 0.3-0.6 (Weiss) units T4 DNA ligase at 14°C . (for "blunt end" ligation). Intermolecular "sticky end" ligations are usually performed at 33-100 $\mu\text{g}/\text{ml}$ total DNA concentrations (5-100 nM total end concentration). Intermolecular blunt end ligations (usually employing a 10-30 fold molar excess of linkers) are performed at 1 μM total ends concentration.

Verification of Construction

Correct ligations for vector construction are confirmed according to the procedures of Young et al., *Nature*, 316:450-452 (1988).

Isolation of Gene Encoding Mammalian Vesicle Membrane Transport Protein

The cDNA of the vesicle membrane transport protein obtained as described above is then used as a probe of a genomic mammalian library to obtain clones containing the complete gene coding sequence of mammalian vesicle membrane transport protein. cDNA probes are constructed from the sequence encoding the chromaffin granule amine transport protein (CGAT) or the synaptic vesicle amine transport protein (SVAT) and used to probe genomic libraries from various mammalian tissues using standard hybridization methods to isolate sequences encoding vesicle membrane transport proteins from other mammalian tissues. Alternatively, sets of synthetic oligonucleotides encoding the protein are used to probe a genomic library. Successful hybridizing clones are sequenced, and those containing the correct nucleotide sequence for the protein are obtained.

Expression

The vesicle membrane protein can be expressed in a variety of systems as set forth below. The cDNA can be excised by suitable restriction enzymes and ligated into procaryotic or eucaryotic expression vectors for such expression.

II. MAMMALIAN VESICLE MEMBRANE TRANSPORT PROTEINS

An aspect of the invention is mammalian vesicle membrane transport proteins purified or isolated from either cells in which the proteins occur naturally or from chimeric cells expressing the protein in a recoverable quantity. Small quantities of such proteins, such as those produced by chimeric cells, can be used to generate protein-specific antibodies for immunoaffinity purification of the protein from cells in which the protein occurs naturally.

When produced from chimeric cells, the mammalian vesicle membrane transport protein can be produced either as a mature protein or as a fusion protein, or can be produced along with a signal sequence in cells capable of processing this sequence for secretion. It can be advantageous to obtain secretion of the protein as this minimizes the difficulties in purification. Cultured mammalian cells are able to cleave and process heterologous mammalian proteins containing signal sequences and to secrete them into the medium (McCormick et al., *Mol. Cell. Biol.* 4:166 (1984)). If secreted, the purification process is simplified, because relatively few proteins are secreted into the medium, and the majority of the secreted protein will, therefore, already be the vesicle membrane transport protein. However, it is also known in the art to purify the protein from membranes of cells in which it is produced in mature or fully processed form.

In one embodiment described below in Example 2, gene transfer was used to express the MPP⁺ toxicity suppression

activity of a vesicle membrane amine transport protein in non-neural cells. This led to the isolation of a cDNA clone that encodes a rat adrenal-specific transport protein, designated herein as chromaffin granule amine transport protein (CGAT protein). The sequence of the cDNA (SEQ ID NO: 1) and the predicted amino acid sequence of the protein encoded by the cDNA (SEQ ID NO: 2) are given in FIG. 1, below. The amino acid sequence of the CGAT protein predicts that its three-dimensional structure has 12 transmembrane domains. The CGAT protein has limited homology to a class of bacterial drug resistance transport proteins.

In another embodiment, as discussed below in Example 3, a probe specific for the DNA encoding CGAT protein was used to screen a bacteriophage λ gt10 rat brainstem cDNA library. Sequencing of the resulting clones resulted in the determination of the nucleotide sequence and protein sequence of the brainstem protein. This protein is designated herein as synaptic vesicle amine transport protein (SVAT protein). The DNA sequence (SEQ ID NO: 3) and corresponding amino acid sequence (SEQ ID NO: 4) for SVAT protein are shown in FIG. 2.

There is substantial homology between the nucleotide and amino acid sequences for CGAT and SVAT proteins, as shown in FIG. 4. In particular, the majority of the amino acid residues of the 12 transmembrane domains are either identical or are closely related by conservative amino acid substitutions. In addition, analysis of the amino acid sequence predicted from the human SVAT cDNA yields 92.5% identity and 96.5% homology to rSVAT proteins. Most of the divergence occurs in the large luminal loop between the first two transmembrane domains, but the human and rat SVAT sequences still show considerable homology in this region.

Accordingly, within the scope of the invention are functional equivalents of CGAT protein or SVAT protein having membrane transport activity. These functional equivalents include, but are not limited to, proteins related to the proteins of FIGS. 1, 2, or 3 (SEQ ID NO: 2, 4, & 13) by one or more conservative amino acid substitutions substantially preserving the structure of the transmembrane domains of the protein. It is a well-established principle of protein chemistry that certain amino acid substitutions, entitled "conservative" amino acid substitutions, can frequently be made in a protein without altering either the conformation or the function of the protein. Such substitutions include, but are not limited to, substituting any of isoleucine (I), valine (V), and leucine (L) for any other of these amino acids, aspartic acid (D) for glutamic acid (E) and vice versa; asparagine (N) for glutamine (Q) and vice versa; and serine (S) for threonine (T) and vice versa. Other substitutions can also be considered conservative depending on the environment of the particular amino acid in the three-dimensional structure of the protein in question. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can be alanine and valine (V). Methionine (M), which is relatively hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R) are frequently interchangeable in locations in which the significant feature of the amino acid residue is its positive charge and the differing pK's of these two basic amino acid residues are not significant. Cysteine (C) can frequently be replaced by serine when cysteine's capacity to form disulfide bonds is either undesirable or unneeded. Still other changes can be considered "conservative" in particular environments when the three-dimensional structure of the protein is taken into account.

Also considered to be within the scope of the present invention are proteins encoded by the nucleic acid sequences

discussed in Section III, below. These nucleic acid sequences include cDNA sequences and genomic DNA sequences.

III. NUCLEIC ACID SEQUENCES

Another aspect of the present invention is nucleic acid sequences coding for a vesicle membrane transport protein as described above. These nucleic acid sequences include: (1) cDNA sequences and (2) genomic DNA sequences.

A. cDNA Sequences

The cDNA sequences coding for vesicle membrane transport proteins according to the present invention comprise substantially pure cDNA encoding the amino acid sequence of FIGS. 1, 2, or 3 (SEQ ID NO: 1, 3, & 12). This cDNA is substantially free of cDNA that does not encode the amino acid sequence of FIGS. 1, 2, or 3 (SEQ ID NO: 1, 3, & 12). The cDNA can have the sequence disclosed in FIGS. 1, 2, or 3 (SEQ ID NO: 1, 3, & 12), or can be related thereto by the degeneracy of the genetic code. The cDNA can be obtained by reverse transcription or chemical nucleotide synthesis.

B. Genomic DNA Sequences

Another aspect of the invention is substantially purified genomic DNA whose exons encode mammalian vesicle membrane transport protein. Such DNA differs from cDNA by possessing intervening non-coding regions or introns. In view of the differences between the adrenal-specific (CGAT) and brain-specific (SVAT) proteins, it is likely that these two subtypes are encoded by different, albeit related, genes, although applicant does not intend to be bound by this theory.

One method of obtaining at least one such gene comprises hybridizing the cDNA described above as a probe to obtain the genomic DNA in the form of DNA hybridizing specifically with the cDNA. Depending on the stringency of the hybridization process, a single cDNA probe can be used to isolate one or more genes for the transport protein. Accordingly, substantially purified genomic DNA coding for a protein that has vesicular amine transport activity and having at least about 60% homology in its exons with at least one of the rat CGAT cDNA or the rat SVAT cDNA is within the scope of the invention, as is a protein thus encoded. This category of genomic DNA includes at least one human gene, as shown in Example 4, below. This human gene has at least about 70% homology in one of its exons with a portion of the cDNA sequence of FIG. 2 extending from about base 220 to about base 540 (FIG. 5) (SEQ ID NO: 5). In fact, this gene corresponds to SVAT, because it includes an exon with a predicted amino acid sequence highly related to the amino acid sequence of the large luminal loop in rSVAT and unrelated to the same region of rCGAT.

In a preferred embodiment described herein, gene transfer was used to express the MPP⁺ toxicity suppression activity of a vesicle membrane amine transport protein in non-neural cells. The gene transferred was a cDNA clone that encodes rat chromaffin granule amine transport protein. The clone was isolated using gene transfer followed by selection in MPP⁺. The cDNA sequence encodes a novel protein with twelve transmembrane domains and homology to a class of bacterial drug resistance transport proteins.

IV. USE OF TRANSPORT PROTEINS AND NUCLEIC ACID SEQUENCES

The rat adrenal chromaffin granule amine transport cDNA of the invention obtained as described herein can be used to isolate the cDNAs or genes encoding human vesicle membrane transport proteins, such as the chromaffin granule amine transport protein, by screening the appropriate human cDNA or genomic library as described above, and to isolate

additional cDNAs or genes encoding vesicle membrane transport proteins from other mammalian tissues using the procedures also set forth above. The chromaffin granule vesicle transport protein is expected to exhibit substantial homology to vesicle transport proteins from other mammalian tissues.

The sequence described herein, combined with the observed homology to the bacterial drug resistance transporters, can be used to design oligonucleotide primers to amplify reverse transcribed cDNA library or other nucleic acids using standard techniques of the polymerase chain reaction. These amplified sequences can then be used to obtain the remainder of the protein coding regions of the cDNAs or genes by screening the appropriate libraries by standard techniques.

The sequences, incorporated in appropriate vectors, can be used to transform suitable host cells for expression of the mammalian vesicle membrane transport proteins. Preferably, to obtain optimum levels of expression, transformants are selected in MPP⁺ to obtain cells expressing the transport protein in a quantity sufficient to render the cells resistant to MPP⁺, or are selected by screening with an antibody specifically binding the transport protein so that a host cell producing a quantity of the transport protein sufficient to react detectably with the transport protein is selected.

A method of producing a mammalian vesicle membrane transport protein according to the present invention can comprise:

- (1) culturing a host cell according to the present invention transformed with an appropriate expression vector;
- (2) using the cultured host cell to express the transport protein; and
- (3) purifying the protein.

The step of using the cultured host cell to express the transport protein can involve altering the culture conditions of the cultured host cell, such as by adding an inducer of an inducible control element to which the gene for the transport protein is operatively linked in the expression vector. The nature of the inducer depends on the particular inducible control element used.

The expressed protein can be purified by conventional methods, such as ion-exchange chromatography, gel filtration chromatography, reverse phase high pressure liquid chromatography, electrofocusing, chromatofocusing, and/or immunoaffinity chromatography, using any readily ascertainable property, such as transport activity.

The mammalian vesicle membrane transport protein, when expressed in functional form in host cells such as CHO cells, can be used to screen compounds, e.g. toxins in addition to MPP⁺, to identify those that when sequestered within intracellular vesicles of various cell types result in cell survival, i.e., whether cytotoxicity of the reagent is reduced by sequestration of the reagent in intracellular vesicles. This may lead to identification of compounds implicated in death of various cell types involved in different diseases. The protein may also be used to screen for compounds that inhibit transport of substances into vesicles, for example to inhibit the transport of norepinephrine or epinephrine, amine transmitters that regulate heart rate and blood pressure. By using transporters expressed in different locations, e.g., from adrenal and brain tissues, it can be possible to screen for compounds having specificity for one transport protein but not the other. Thus, a compound can be obtained that inhibits the transport protein from adrenal tissue, but not the transport protein from brain tissue and so provide a way to reduce blood pressure without causing

lethargy or depression. Reserpine, like other anti-hypertensive medications, produces lethargy and depression. By identifying a compound that inhibits amine transport in the adrenal gland but not the brain, it may be possible to eliminate this side effect. The recombinant protein can be used to screen for such compounds. In addition, the vesicle membrane transport protein can also be used to screen for compounds that inhibit central nervous system function but not adrenal transport. These compounds might be very useful as tranquilizers, anxiolytic or anti-psychotic drugs. This screening process is facilitated by the use of tissue-specific subtypes of the mammalian vesicle membrane transport protein, such as the adrenal-specific CGAT protein and the brain-specific SVAT protein.

This screening process is not limited to the determination of cell survival. It can be carried out by contacting cells containing a nucleotide sequence encoding the transport protein with a compound suspected of selectively inhibiting or activating the activity of a vesicle membrane transport protein, and then determining the activity of the transport protein within the cells contacted with the compound to determine whether the compound selectively inhibits or activates the activity of the transport protein.

This screening process can be performed by qualitative or quantitative assays of membrane transport, or, alternatively, by binding assays to determine the affinity of the compound for the protein. Such binding assays can be performed by: (1) incubating a substantially purified preparation of mammalian vesicle membrane transport protein with a compound suspected of being an inhibitor; and (2) comparing the binding by the transport protein to an amine normally bound by the transport protein of the preparation in the presence and absence of the compound to determine whether the compound has inhibitory activity.

One preferred version of a qualitative assay, described in Example 1, below, detects uptake and cellular compartmentalization of loaded dopamine in cells grown on polylysine-coated glass coverslips. After incubation of the cells in glyoxylic acid-MgCl₂, uptake of the dopamine is detected by fluorescence viewed through a fluorescence microscope.

One preferred version of a quantitative assay, described in Example 1 below, measures the uptake of labeled dopamine by the membrane fraction resulting from cellular homogenates. The quantitative assay is performed on transfected cells that are selected in MPP⁺ or have been screened with antibodies so that cells with a higher level of expression of the transporter are used. The quantity of label bound is measured after filtration of the assay mixture. Preferably, the label is a radioactive label. By varying the dopamine concentration, the K_m for dopamine transport can be determined. This can be done by standard graphic kinetic techniques such as the Lineweaver-Burke plot. The K_i for inhibition of dopamine uptake by serotonin, epinephrine, or norepinephrine can also be determined by similar kinetic techniques.

The mammalian vesicle membrane transport protein and nucleotide coding sequences have various diagnostic and therapeutic applications. For example, the ability of the protein to sequester the toxin MPP⁺ implicated in Parkinson's disease provides the ability to protect cells from this toxin. In addition, the transport protein coding sequences, when transfected into a suitable host cell, can be used to screen for drugs that affect the functional expression of the protein. Such drugs may either upregulate or downregulate the expression of the transport protein or induce post-translational modifications that alter activity of the expressed protein.

DNA or RNA sequences coding for hCGAT or hSVAT can be used as probes to detect chromosomal abnormalities. As shown below in Example 5, the gene for human CGAT occurs on chromosome 8 at 8p21.3. At least one report exists of a disease, hereditary spherocytosis, due to a deletion that may include the gene for CGAT. Probes corresponding to this gene may be useful in detection of this chromosomal abnormality by hybridization, and polymerase chain reaction (PCR) amplification procedures or other nucleic acid amplification procedures involving initiation from delimited primers can be used to determine the extent of the deletion.

Similarly, the human gene for SVAT is located on chromosome 10 at 10q25, and deletions from a point surrounding this region to end of the chromosome have appeared in the literature and the phenotype associated with this deletion includes dysmorphic features and severe mental retardation. Accordingly, diagnostic techniques involving hybridization with labeled probes can be used to detect these conditions as well. A suitable procedure can comprise:

- (1) hybridization of a labeled single-stranded nucleic acid sequence of sufficient length to hybridize specifically to chromosomal DNA and whose sequence is derived from the human SVAT gene to human chromosomal DNA; and
- (2) detecting the hybridized labeled nucleic acid sequence to determine the presence or absence of an abnormality in human chromosome 10.

The labeled single-stranded nucleic acid sequence is preferably from the large lumenal loop to allow the method to distinguish between hSVAT and hCGAT. Typically, the labeled nucleic acid sequence is at least about 10 nucleotides in length, more typically at least 50 nucleotides, preferably at least 100 nucleotides.

In addition, the vesicle membrane transport coding sequences can be used to enhance sequestration of MPP⁺ into mammalian cells to treat Parkinson's Disease. To carry out such treatment, the gene or cDNA encoding the vesicle transport protein must be placed in cells located in the midbrain so as to cause expression of the gene in vivo. For example, an effective number of cells in the midbrain are transfected with vectors carrying the gene encoding the vesicle transport protein, such as recombinant herpes simplex virus vectors (see Dobson et al., *Neuron* 5:353-360 (1990); Geller and Freese, *Proc. Natl. Acad. Sci. USA* 87:1149-1153 (1990) and Preston et al., PCT Application Ser. No. WO 91/02788, "Herpes Simplex Virus Type I Mutant"), to produce sufficient amounts of the vesicle membrane transport protein in vivo to permit the cells of the midbrain to sequester toxins such as MPP⁺.

Alternatively, the nucleotide sequence encoding the mammalian vesicle transport protein of the invention can be operably linked to control sequences that enhance or decrease the expression of the protein. This construct can then be transferred or transfected via an appropriate vector, for example a retroviral vector into suitable host cells, for example primary fibroblasts or neural cells, which are then introduced into a mammal to express and/or secrete the vesicle membrane protein (see Gage et al., U.S. Pat. No. 5,082,670 (1992)). For example, control sequences that increase the expression of the vesicle transport protein of the invention can be used to enhance sequestration of toxic molecules into cells to confer resistance in a mammalian subject to the toxic molecules.

Vesicle membrane transport proteins that transport other compounds, such as acetylcholine and various neurotoxins including glutamate, can be used to modulate the uptake and storage of such compounds to combat the deleterious effects of accumulations of these compounds.

An important use of the vesicle membrane transport proteins of the invention, particularly those that transport amines, is the treatment of psychiatric diseases such as affective disorders including bipolar disease and Schizophrenia and other diseases where there is an imbalance of amines, including norepinephrine, dopamine and serotonin. For treatment, the activity of the vesicle membrane transport protein is increased, for example by modifying expression of the protein using vectors carrying enhancers of the gene or cDNA encoding the vesicle membrane transport protein to transfect cells in the brain.

In addition, the mammalian vesicle membrane transport proteins can be used to diagnose susceptibility to various diseases resulting from imbalances of compounds in vivo. Thus, the protein or nucleotide sequences coding for it can be used to identify subjects having reduced activity of the protein or reduced levels of RNA encoding the protein. This is accomplished using known polymorphisms in the nucleotide sequence that confer alterations in activity. The genes encoding the vesicle membrane transport proteins may prove useful to determine whether the genes are linked to various psychiatric diseases, and this information can then be used to design drugs that increase or decrease transport of compounds across the vesicle membranes to treat the diseases resulting from imbalances in these compounds.

In addition, selection of cells carrying mutations in the gene or cDNA encoding the vesicle membrane transport protein in the presence of MPP⁺ and reserpine or tetrabenazine or other compounds can be used to define the site of action of these other compounds. For example, the cDNA encoding the transport protein can be altered by standard procedures such as site-directed mutagenesis or saturation mutagenesis, for example using mutagenic bacteria (Fowler et al., *Mol. Gen. Genet.* 133:179-191 (1991); Silhavy et al., "Experiments with Gene Fusions," Cold Spring Harbor Press, 1984, pp. 75-78). The mutagenized cDNA is used to transfect cells for selection in the presence of MPP⁺ and a compound capable of inhibiting the transport protein. These procedures will provide information about the site of action of drugs on the transport protein. This information, in turn, can be used to design compounds with desired properties such as greater activity or improved therapeutic abilities. In addition, variant vesicle transport proteins can be obtained that are not inhibited by drugs such as reserpine and tetrabenazine.

V. PHARMACEUTICAL COMPOSITIONS AND ANTIBODIES

Another aspect of the invention is antibodies specific for the vesicle membrane transport proteins and pharmaceutical compositions incorporating the proteins.

The vesicle membrane transport protein can be used to prepare antibodies, including polyclonal antibodies that bind to purified or recombinant vesicle transport protein or peptides. These antibodies can be used to purify the vesicle membrane transport protein in larger quantities. For example, the transport protein can be purified by fixing the antibody to a solid support and reacting the antibody fixed to the solid support with a sample containing the transport protein to bind the transport protein to the antibody. Alternatively, the antibody can be labeled with a detectable label, reacting the antibody labeled with the detectable label with a sample containing transport protein to bind the transport protein to the antibody, thereby forming an antigen-antibody complex, and separating the antigen-antibody complex from other proteins present in the sample. In either case, the protein can then be dissociated from the antibody by standard techniques, such as high salt, change of pH, or low concentrations of chaotropic agents.

The purified protein can then be used, for example, to screen for compounds capable of blocking binding of substances such as reserpine to the purified transport protein (Stern-Bach et al., *J. Biol. Chem.* 265:3961-3966 (1990)). Such screening can be used to identify compounds that bind to the plasma membrane transport protein but not to the vesicle membrane transport protein for selectively blocking the binding to one or the other transport protein.

Similarly, tissue-specific subtypes of purified vesicle membrane transport protein can be used to identify tissue-specific inhibitors by determining the degree of inhibition caused by the inhibitor for a first tissue-specific subtype and for a second tissue-specific subtype and comparing the degree of inhibition. This procedure can be used, for example, to identify an inhibitor of the adrenal transport protein that does not affect the brain transport protein. This should assist in the development of new blood pressure medications that do not exhibit side effects on the central nervous system, or, conversely, new tranquilizers, anxiolytics, or antidepressants that do not exhibit systemic side effects.

Monoclonal antibodies reactive with vesicle membrane transport protein can be produced by hybridomas prepared using known procedures, such as those introduced by Kohler and Milstein (see Kohler and Milstein, *Nature*, 256:495-97 (1975)), and modifications thereof, to regulate cellular interactions.

These techniques involve the use of an animal which is primed to produce a particular antibody. The animal can be primed by injection of an immunogen (e.g. the vesicle membrane transport protein) to elicit the desired immune response, i.e. production of antibodies from the primed animal. A primed animal is also one which is expressing a disease. Lymphocytes derived from the lymph nodes, spleens or peripheral blood of primed, diseased animals can be used to search for a particular antibody. The lymphocyte chromosomes encoding desired immunoglobulins are immortalized by fusing the lymphocytes with myeloma cells, generally in the presence of a fusing agent such as polyethylene glycol (PEG). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques; for example, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653, Sp2/0-Ag14, or HL1-653 myeloma lines. These myeloma lines are available from the ATCC, Rockville, Md.

The resulting cells, which include the desired hybridomas, are then grown in a selective medium such as HAT medium, in which unfused parental myeloma or lymphocyte cells eventually die. Only the hybridoma cells survive and can be grown under limiting dilution conditions to obtain isolated clones. The supernatants of the hybridomas are screened for the presence of the desired specificity, e.g. by immunoassay techniques using the vesicle membrane transport protein that has been used for immunization. Positive clones can then be subcloned under limiting dilution conditions, and the monoclonal antibody produced can be isolated.

Various conventional methods can be used for isolation and purification of the monoclonal antibodies so as to obtain them free from other proteins and contaminants. Commonly used methods for purifying monoclonal antibodies include ammonium sulfate precipitation, ion exchange chromatography, and affinity chromatography (see Zola et al., in *Monoclonal Hybridoma Antibodies: Techniques and Applications*, Hurell (ed.) pp. 51-52 (CRC Press, 1982)). Hybridomas produced according to these methods can be propagated *in vitro* or *in vivo* (in ascites fluid) using techniques known in the art (see generally Fink et al., *Prog. Clin. Pathol.*, 9:121-33 (1984), FIG. 6-1 at p. 123).

Generally, the individual cell line can be propagated *in vitro*, for example, in laboratory culture vessels, and the culture medium containing high concentrations of a single specific monoclonal antibody can be harvested by decantation, filtration, or centrifugation.

In addition, fragments of these antibodies containing the active binding region reactive with the vesicle membrane transport protein, such as Fab, F(ab')₂ and FV fragments can be produced. Such fragments can be produced using techniques well established in the art (see e.g. Rousseaux et al., in *Methods Enzymol.*, 121:663-69, Academic Press (1986)).

Polyclonal antibodies can be produced, for example polyclonal peptide antibodies as described by Hirayama et al., in *Am. J. Physiol.* 261:C296-C304 (1991). Briefly, peptides are synthesized, e.g. as described by Kent and Clark-Lewis, in *Synthetic Peptides in Biology and Medicine*, Amsterdam, Elsevier, p. 29-57 (1985), and are purified using reverse-phase high-performance liquid chromatography on a preparative C₈ column in a gradient of 17.5-32.5% acetonitrile with 0.1% trifluoroacetic acid (TFA). The purity of the product is verified by isocratic elution on a C₁₈ column in 25.5% acetonitrile and 0.1% TFA and by mass spectroscopy before lyophilization. Immunization can be accomplished by coupling to keyhole limpet hemocyanin. Polyclonal antibodies are then raised in rabbits following standard procedures using the peptides as immunogen. These procedures permit the production of antibodies that bind to defined regions of the vesicle membrane transport protein amino acid sequence, using peptides or portions of peptides of the vesicle membrane protein as immunogen.

The compositions and antibodies of the invention are administered *in vivo* into a mammal using conventional modes of administration which include, but are not limited to intravenous, oral, subcutaneous, intraperitoneal, and intralymphatic. The compositions can be administered for gene therapy.

The pharmaceutical compositions of the invention comprising inhibitors of amine transport, or antibodies, can be in a variety of dosage forms which include, but are not limited to, solid, semi-solid and liquid dosage forms such as tablets, pills, powders, liquid solutions or suspensions, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the therapeutic application.

Conventional pharmaceutically acceptable carriers for the compositions may include those known in the art such as serum proteins including human serum albumin, buffer substances such as phosphates, water or salts or electrolytes.

The most effective mode of administration and dosage regimen for the compositions of this invention depends upon the patient's health and response to treatment and the judgment of the treating physician. Accordingly, the dosages of the compositions should be titrated to the individual patient.

The following examples are presented to illustrate the present invention and to assist one of ordinary skill in the art in making and using the invention. The examples are not intended in any way to otherwise limit the scope of the invention.

EXAMPLE 1

Suppression of MPP⁺ Toxicity by Gene Transfer of Vesicle Membrane Amine Transport Protein

This example describes the isolation of a cDNA clone extremely resistant to the toxin MPP⁺ using rat pheochromocytoma PC12 cells as a source of a cDNA expression library transfected into MPP⁺-sensitive CHO fibroblasts.

Cell culture

All cells were maintained in a humidified incubator at 37° C. in 5% CO₂. PC12 cells were grown in Dulbecco's modified Eagle's medium containing 10% horse serum and 5% fetal calf serum. CHO cells in Ham's F12 medium with 10% fetal calf serum. MPP⁺ (Research Biochemicals, Inc., Natick, Mass.) was dissolved in Ham's F12 medium without serum at a concentration of 20 mM, passed through a 0.22 μm filter, then added at the appropriate concentration to the medium without serum. Rotenone (Sigma Chemical Co., St. Louis, Mo.), oligomycin (Sigma) and dopamine (Sigma) were similarly dissolved in medium, filtered and added to the cultures. Reserpine (Sigma) was dissolved in dimethylformamide before adding to the medium.

RNA preparation and cDNA library construction

PC12 cells were grown to relatively high density, mechanically dislodged from the plate, sedimented in a clinical centrifuge and homogenized in guanidinium isothiocyanate as described by Chirgwin et al., *Biochem.* 18:5294-5299 (1979). Briefly, a guanidinium thiocyanate stock was prepared by mixing guanidinium thiocyanate with sodium N-lauroylsarcosine, sodium citrate, 2-mercaptoethanol and antifoam A (Sigma, St. Louis, Mo.). The ionized water was added and the pH adjusted to 7.0 with 1.0N NaOH. The samples were homogenized into guanidinium thiocyanate stock solution in a homogenizer for 30-60 seconds at full speed. The homogenates were centrifuged for 10 min. at 8,000 rpm at 10° C. to sediment particulate material. The supernatants were decanted into a flask and mixed with 0.025 volume (relative to the original volume of homogenizing buffer) of 1M acetic acid to lower the pH from 7 to 5 and 0.75 volume of absolute ethanol. The flask was capped, shaken thoroughly, and placed at -20° C. overnight to precipitate nucleic acid. The material was sedimented by centrifugation for 10 min. at -10° C. and 6,000 rpm. The tubes were drained of supernatant and any material that did not form a firm pellet. The pellet was then resuspended by vigorous shaking in 0.5 volume (relative to the original volume of homogenization buffer) of buffered guanidinium chloride stock solution (pH 7.0, buffered with 0.025 volume of 1M sodium citrate, pH 7.0, 5 mM in dithiothreitol or dithioerythritol). If necessary, the samples were briefly warmed in a 68° C. water bath to ensure complete dispersion. RNA was reprecipitated by adding (relative to the amount of guanidinium chloride) 0.025 volume of 1M acetic acid and 0.5 volume of ethanol. The solution was kept for at least 3 hr. at -20° C. and centrifuged as before. A final reprecipitation from guanidinium chloride was performed in the same way, with a further halving of the total volume. This reprecipitated material was centrifuged for only 5 min at 6,000 rpm. The final pellets were dispersed in ethanol at room temperature, triturated if necessary to extract excess guanidinium chloride, and again centrifuged for 5 min at 6,000 rpm. Ethanol was removed from the pellet by a stream of nitrogen, and the RNA was dissolved by vigorous shaking in sterile water. The solution was centrifuged for 10 min at 13,000 rpm and 10° C. to sediment insoluble material. The supernatants containing the RNA were decanted and saved, while the insoluble material was reextracted twice with the sterile water, followed by centrifugation for 10 min at 13,000 rpm and 10° C. The combined aqueous solution was mixed with 0.1 volume of 2M potassium acetate, pH 5, and precipitated with ethanol. After centrifugation, the pellets were washed with ethanol, dried with nitrogen, and dissolved in sterile water. The RNA was separated by centrifugation through cesium chloride, resuspended in 10 mM Tris/1 mM EDTA containing 0.1%

SDS, extracted with phenol, then chloroform, and precipitated twice in ethanol. Oligo-dT cellulose was used to isolate the polyA⁺ fraction of RNA as described by Aviv and Leder, *Proc. Natl. Acad. Sci. USA* 69:1408-1412 (1972). Briefly, the RNA was dissolved in application buffer containing 0.01M Tris-HCl, pH 7.5, 0.5M KCl, and was applied to an oligo-dT cellulose column previously washed with application buffer. The non-absorbed material was eluted by continued washing with the application buffer. The material retained by the column was eluted in two steps with buffers of reduced ionic strength. The first elution buffer contained 0.01M Tris-HCl, pH 7.5, 0.1M KCl, the second 0.01M Tris-HCl, pH 7.5. The material eluted by the two elution steps with buffers of reduced ionic strength was combined and precipitated by the addition of potassium acetate and two volumes of ethanol. The first strand of cDNA was synthesized from 5 μg polyA⁺ RNA using oligo-dT as primer and avian myeloblastosis virus reverse transcriptase. The second strand was synthesized with RNase H and *E. coli* DNA polymerase and, after rendering the ends of the double-stranded cDNA blunt with T4 DNA polymerase, BstXI linkers were added as described by Gubler and Hoffman, *Gene* 25:263-269 (1983); and Aruffo and Seed, *Proc. Natl. Acad. Sci. USA* 84:8573-8577 (1987). Briefly, blunt-end ligation was performed on phosphorylated oligonucleotide linkers in a reaction mixture containing 6 mM Tris-HCl, pH 7.5, 6 mM MgCl₂, 5 mM NaCl, 0.35 mg/ml bovine serum albumin, 7 mM 2-mercaptoethanol, 0.1 mM ATP, 2 mM dithiothreitol, 1 mM spermidine, and 400 units of T4 DNA ligase in a 0.3-ml reaction mixture at 15° C. overnight. Free linker and cDNA fragments less than 0.8-1.5 kb in length were removed by electrophoresis through 5% acrylamide under nondenaturing conditions, and the larger size-selected material electroeluted and precipitated twice with ethanol. The cDNA was then ligated into the CDM8 vector from which the BstXI stuffer fragment has been removed, and transformed into bacteria by electroporation as described by Dower et al., *Nucl. Acids. Res.* 16:6127-6145 (1988). Briefly, the exponential decay pulses were generated by a Gene Pulser apparatus (Bio-Rad Laboratories, Richmond, Calif.) set at 3 or 25 μF and from 0.2 to 2.5 kV. The output of the pulse generator was directed to a pulse controller unit (Bio-Rad) containing a high power, 20-ohm resistor in series with the sample, and a selection of resistors of 100 to 1,000 ohms in parallel with the sample. The effective resistance placed in parallel with the electrodes is much lower than that of the sample, and determines the time constant of the pulse (for example, 200 ohms with a 25 μF capacitor gives a 5 msec time constant. Electrode gaps of either 0.15 cm with a special "minielectrode", or 0.2 cm with a small gap Potter-type cuvette (Bio-Rad) were used. These electrode configurations provided field strengths of up to 16.7 kV/cm and 12.5 kV/cm. The concentrated cells were thawed at room temperature and placed on ice. The cells (40 μl) were transferred to a cold, 1.5 ml polypropylene tube; 1 to 2 μl of DNA solution (in a low ionic strength medium such as TE) was added to give a final concentration of from 10 pg/ml to 7.5 μg/ml, and this suspension was mixed vigorously by flicking the tube. The cell-DNA mixture was placed between the chilled electrodes, the electrode assembly or cuvette placed in the safety chamber, and the appropriate pulse applied. Following the pulse, the cells were immediately removed from the electrodes and mixed in 25 to 50 volumes of outgrowth medium (2% Bacto-Tryptone, 0.5% Bacto-Yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl₂, 10 mM MgSO₄, 20 mM glucose) in a 17×100 mm polypropylene tube. The samples were incubated for 1 hr at

37° C. At the end of the expression period, the cells were diluted and plated on agar containing the appropriate antibiotic to screen for transformants.

Gene transfer

Library plasmid DNA was prepared by alkaline lysis, sedimented twice through CsCl, then extracted with phenol and precipitated twice in ethanol. With the RSV-neo plasmid as a selectable marker for stable transformation, the DNA was reprecipitated under sterile conditions and transfected using a modified calcium-phosphate procedure as described by Chen and Okayama, *Mol. Cell. Biol.* 7:2745-2752 (1987) with the buffer BES (N,N-bis-(2-hydroxyethyl)-2-aminoethanesulfonic acid) at pH 6.95 and 3% CO₂ and the plasmid expression vector CDM8 (Aruffo and Seed, supra, supplied by Drs. Aruffo and Seed, Harvard University, Boston, Mass.).

MTT assay

Differential susceptibility to the respiratory complex I inhibitors MPP⁺ and rotenone was shown by measuring toxicity by measuring general cell dehydrogenase activity with reduction of a tetrazolium dye (MTT) in a spectrophotometric plate assay as described by Hansen et al., *J. Immunol. Methods* 119:203-210 (1989). Briefly, a stock solution of MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide) was dissolved at a concentration of 5 mg/ml in sterile phosphate buffered saline at room temperature. To each well was added 25 µl of the stock solution. After two hours of incubation at 37° C., 100 µl of extraction buffer (20% w/v of SDS in a solution of 50% each dimethylformamide and demineralized water with the pH adjusted to 4.7 with acetic acid and HCl). After an overnight incubation at 37° C., the optical densities at 570 nm were measured using a multiscanner, employing the extraction buffer as the blank. This assay was carried out in triplicate on 96-well plates (NUNC, Denmark). MPP⁺ and rotenone at the concentrations indicated in FIG. 6a and 6b, respectively, was added to wild-type and MPP⁺-resistant CHO cells at approximately 50% confluence, and toxicity was assayed after 2-3 days by measuring general cell dehydrogenase activity with the reduction of the tetrazolium dye MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide). After addition of the dye at a final concentration of 1 mg/ml, the cells were incubated for two hours at 37° C. and the formazan product was solubilized in 10% sodium dodecyl sulfate (SDS). The plates were maintained at 37° C. for an additional twenty hours and the optical density was determined in an ELISA plate reader at a test wavelength of 570 nm, a reference wavelength of 630 nm and a calibration setting of 1.99. All determinations were performed in triplicate and expressed as the mean, with the error bars representing standard deviation (FIG. 6a and 6b).

Oxygen consumption

The cells were trypsinized, resuspended in Krebs-Ringer buffer, counted in a hemocytometer and respiration was determined on different numbers of cells in a glass chamber with a Clark oxygen electrode as described by Denton and Howard, supra.

Catecholamine uptake

Uptake of radiolabelled MPP⁺ was determined by distributing cells into a 24-well plate (NUNC) in the presence of serum, washing them the next day in serum-free medium and then incubating them in serum-free medium containing [³H]-MPP⁺ (NEN, Wilmington, Del.). At the end of incubation for varying intervals up to several days, the cells were washed three times in serum-free medium without radiolabelled drug, solubilized in 1% SDS and an aliquot counted in Ecolite (ICN Pharmaceuticals, Irvine, Calif.). All mea-

surements were determined in duplicate or triplicate and normalized to protein content.

A method was devised to measure intracellular compartmentalization of exogenously loaded catecholamines.

Uptake and cellular compartmentalization of loaded dopamine were performed by growing cells on poly-lysine-coated glass coverslips. After cell attachment, the medium was replaced with standard medium plus serum that contains 0.5-1.0 mM dopamine and incubated for an additional 12-24 hours. The cells were then washed three times in 0.1M sodium phosphate, pH 7.4, and incubated at 4° C. in 2% glyoxylic acid/0.5 mg% MgCl₂, pH 4.9-5.0 for three minutes as described by de la Torre, *J. Neurosci. Meth.* 3:1-5 (1980). Briefly, excess solution is wiped off the bottom and edges of the slide using absorbent paper, taking care not to touch the section. The coverslips were then drained thoroughly, dried in air at 45° C. heated to 80° C. for five minutes, inverted over mineral oil on glass slides and examined under fluorescence using the appropriate filters as described by Knigge et al., *Brain Res.* 120:393-405 (1977). Briefly, the preparations were infiltrated with warm immersion oil and examined in a Leitz MPV2 microspectrofluorometer equipped with Schoffel excitation and emission grating monochromators, photomultipliers, and ratio computing circuitry. Cells were scanned with either monochromatic light (370 nm) or narrow band excitation (S405, BG3) light for the purpose of identifying fluorescence within cells. Ploem illumination was used in combination with a X 63 or X 100 oil immersion objective. The high concentration of dopamine used to load the cells enters through nonspecific low affinity systems, and thus bypasses the need for high affinity uptake. Microscopic examination of the loaded cells using the induced fluorescence technique permits direct visualization of the cellular distribution of exogenously supplied dopamine.

A quantitative assay to measure dopamine uptake was performed as follows. Mpp^{res} transformants and wild type CHO cells were homogenized at 0.01 mm clearance in cold 0.32M sucrose-10 mM HEPES-KOH, pH 7.4 (SH) containing 5 mM MgEGTA, 1 µg/ml leupeptin and 0.2 mM diisopropylfluorophosphate, and the cell debris removed by centrifugation at 3500x for 5 minutes. An aliquot containing 100 to 150 µg protein from this low speed supernatant was then added to 200 µl SH containing 4 mM KCl, 4 mM MgSO₄, 5 mM ATP, 44 nM [³H]dopamine, and, if inhibition of uptake is to be measured, the inhibitory compound. Incubation was performed at 29° C. for the time period indicated (from 2 minutes to 30 minutes). The assay was terminated by dilution in cold assay buffer followed by filtration through 0.2 µm Supor™ 200 membranes (Gelman, Ann Arbor, Mich.) and the bound radioactivity was measured. The cells used for the quantitative assay are selected in MPP⁺ or screened with antibody to ensure that they express the transporter at high levels, as it is difficult to measure activity in this assay at low levels of transporter expression.

To determine whether the transfer of DNA sequences from PC12 cells into CHO cells could be used to generate stable CHO transformants resistant to MPP⁺, it was first necessary to determine the spontaneous rate of resistance under different culture conditions. Untransfected CHO cells selected in 500 µM MPP⁺ showed different patterns of toxicity depending on cell density. At high density (more than 75% confluence), all of the cells died within 24 hours, with no possibility for subsequent growth. At low density (less than 25% confluence), the cells stopped growing, gradually acquiring refractile cytoplasmic inclusions, swell-

ing and eventually detaching from the plate after several weeks. Selection of one million CHO cells at intermediate density (25–50% confluence) in 500 μM MPP⁺ gave rise to 5–10 small colonies after one month. However, these cells contained particulate inclusions characteristic of MPP⁺ toxicity, and grew very slowly even when maintained at low density. At moderate density, the cells degenerated further and detached from the plate. Thus, untransfected CHO cells were shown to acquire little resistance to 500 μM MPP⁺ during selection for over two months.

Using the above methods, the PC12 cDNA library constructed in the plasmid expression vector CDM8 was transfected into CHO cells. Using the selectable marker RSV-neo (Walker, Weizmann Institute, Israel), two hundred to 500,000 independent stable transformants were obtained after selection in the neomycin analogue G418 at 400 $\mu\text{g}/\text{ml}$ effective dose. These cells were then selected at 40% confluence in 1 mM MPP⁺. Virtually all of the cells developed toxicity within one week, and many detached from the plate by three weeks, with no evidence of healthy cells at that time. However, at four weeks, a colony of cells without refractile inclusions appeared and rapidly covered the plate. The apparently normal growth of these cells in the presence of 1 mM MPP⁺ stands in marked contrast to all of the resistant clones obtained without transfection, which grew very slowly if at all. Using the MTT assay to measure cell toxicity as described above, a steep dose-response curve to MPP⁺ in wild-type cells was observed and a relative lack of toxicity in the selected resistant cell clone (FIG. 6a). However, at 1 mM MPP⁺, the resistant cells did show some toxicity, particularly when the assay was performed at higher cell density.

Because changes in cell density and presumably growth rate were observed to affect MPP⁺ toxicity, it was important to determine whether the transfected clone's resistance arose from a change in growth rate. However, repeated determination of growth rate in the resistant clone in the absence of MPP⁺ showed no difference from wild-type CHO cells. In the presence of MPP⁺, the resistant clone did grow slightly more slowly than untreated wild-type cells, particularly at higher densities.

Resistance to MPP⁺ could derive from improved ability of the cell to compensate for inhibition of respiration, such as by an increased dependence on glycolysis (Denton and Howard, *J. Neurochem.* 49:622–630 (1987); and Reinhard et al., *J. Neurochem.* 55:311–320 (1990)). To determine the role of such compensatory mechanisms, oxygen consumption was measured to examine the primary site of MPP⁺ action in the respiratory chain. If a compensatory mechanism was responsible for resistance, MPP⁺ would be expected to inhibit oxygen consumption in both wild-type and resistant cells. As shown in FIG. 7a, 500 μM MPP⁺ dramatically inhibits oxygen consumption as early as 12 hours after exposure in wild-type cells. However, in the resistant transfected cells, MPP⁺ does not detectably affect oxygen consumption (FIG. 7b). Thus, although improved ability to compensate for respiratory injury remains a possible, additional mechanism for resistance to MPP⁺, the principal mechanism for resistance in the clone appears to reside either in the primary site of action of the drug in the respiratory chain, or in its metabolism and distribution by the cell.

To determine whether an alteration in the process of respiration was responsible for the resistance to MPP⁺, the effects of other known respiratory inhibitors were examined. Oligomycin inhibits complex III of the respiratory chain, and demonstrated the same pattern of toxicity in wild-type

and in resistant transfected CHO cells. This was consistent with the previous finding that the mechanism of resistance in these cells does not derive from an improved ability to compensate for respiratory injury. Rotenone inhibits complex I of the respiratory chain and competes with MPP⁺ for binding to its presumed chain of action (Krueger et al., supra, and Ramsay et al., supra). Thus, if the resistance were due to a change in the site of action, the transfected cells should also show resistance to rotenone. Repeated experiments have demonstrated no substantial resistance to rotenone in the transfectant (FIG. 7b). However, rotenone is considered to have its selected effect on respiration at relatively low doses, and an additional, less specific effect at higher doses. Toxicity using the MTT assay showed a biphasic pattern in CHO cells (FIG. 6b), but in no dose range was there a clear difference of the resistant cells from wild-type. Because the primary site of drug action was unaltered by these criteria, the mechanism of resistance in the transfected cells appears specific for MPP⁺, suggesting a role for altered drug metabolism or distribution.

Because CHO cells lack a system for the high affinity uptake of catecholamines, resistance to MPP⁺ cannot be due to loss of this system. Although a different type of activity might be responsible for drug export, the uptake of 25 nM [³H]-MPP⁺ over one to 48 hours at 37° C. showed no difference between wild-type and resistant cells, providing no support for an active efflux mechanism. Thus, the cells show little evidence for an alteration in the primary site of drug action or for active export.

Because previous studies suggested a potential mechanism for resistance to MPP⁺ related to changes in cellular drug distribution and that the MPP⁺ uptake mechanism might protect against the toxin by sequestering it in the granules, and because PC12 cells express reserpine-sensitive vesicular uptake of amines, it was proposed that transfer of the reserpine-sensitive MPP⁺ uptake activity was responsible for the resistance to MPP⁺ toxicity observed in the CHO transfectant. Therefore, the toxicity of MPP⁺ to transfected cells in the presence of 1 μM reserpine was examined. Dramatic reversion to wild-type CHO sensitivity was observed (FIG. 7a). To demonstrate that reserpine does not affect a mechanism present in wild-type CHO cells, the effect of reserpine on wild-type cells treated with lower concentrations of MPP⁺ to which they are normally sensitive is shown in FIG. 7b. The only changes occurred at the top of the steepest section of the dose-response curve, which typically shows the greatest random fluctuation and standard deviation in this assay (see FIG. 6). Thus, reserpine shows very little reproducible effect on MPP⁺ toxicity in wild-type cells. These results suggest that the transfected cell clone survived selection in MPP⁺ because the cells express a vesicular amine uptake activity, presumably derived from PC12 cells, that effectively sequesters the toxin from its primary site of action in mitochondria.

For direct observation of whether the resistant cells expressed an intracellular amine transport activity, it was necessary to circumvent the absence of a high affinity plasma membrane transport system in the CHO transfectant. Neuronal cells that synthesize amines usually express both plasma membrane and synaptic vesicle catecholamine transport activity. Detection of specific uptake requires amines radiolabelled to high specific activity. Using either labelled MPP⁺ or catecholamine in the 10 to 100 nM range, it is possible to detect both specific plasma membrane and vesicular uptake in neuronal cultures. However, if the cell lacks a high affinity plasma membrane transporter, catecholamines at these low concentrations will not effectively

enter the cell. Thus, it was necessary to use higher concentrations of catecholamine to observe intracellular transport in the transfected CHO cells. If radiolabelled MPP⁺ is diluted with unlabelled MPP⁺ to obtain the concentrations required for significant low affinity uptake, the amount of radioisotope entering the cell is too low to measure reliably.

Using the method described above to measure intracellular compartmentalization of exogenously loaded catecholamines, wild-type CHO cells demonstrated a diffuse, ground-glass pattern of cytoplasmic catecholamine fluorescence (FIG. 9a). In contrast, the resistant cells showed a very different pattern, with intense accumulation of catecholamine in a perinuclear location (FIG. 9b). The cytoplasm also contained scattered punctate fluorescent stain, but less general cytoplasmic fluorescence than wild-type cells, presumably reflecting reduced access of MPP⁺ to mitochondria and so accounting for the differences in sensitivity. To determine whether the pattern of catecholamine accumulation in the transfectant was inhibited with reserpine, the same histofluorescence assay was performed using reserpine, and reversion to the wild-type pattern of catecholamine accumulation was observed (FIG. 9c). These results confirm that the mechanism of MPP⁺ resistance in the transfected cells involves reserpine-sensitive sequestration of the drug within an apparently distinct intracellular compartment. In contrast to the results described by Reinhard et al., *Proc. Natl. Acad. Sci. USA* 84:8160–8164 (1987) which show that reserpine can potentiate the depletion of amines and reduction in tyrosine hydroxylase by MPP⁺, the results presented herein demonstrate that the rat adrenal chromaffin granule transport protein actually modulates MPP⁺ toxicity to the cell, using cell death as the endpoint.

The above results demonstrate the use of gene transfer to confer substantial resistance in recipient cells to the toxin MPP⁺. The reversal of MPP⁺ resistance by reserpine and the intracellular accumulation of loaded dopamine indicate that resistance to the toxin arises from expression of a vesicle membrane amine transport protein. While not wishing to be bound by any theory, it is believed that this transport activity reduces the cytoplasmic level of toxin by sequestering MPP⁺ inside an intracellular compartment, thereby decreasing the amount of drug available to enter mitochondria and inhibit respiration. This demonstration that the transport activity suffices to protect cells from MPP⁺ toxicity implicates the transport protein as a major determinant of resistance among aminergic cell populations. Moreover, this activity confers resistance to MPP⁺ even in a non-neuronal cell line that lacks the synaptic vesicles in which such a transporter normally functions.

The above results implicate the balance between plasma membrane and vesicular uptake of catecholamines as a crucial determinant of MPP⁺ toxicity. Although a number of aminergic populations accumulate the toxin by high affinity plasma membrane uptake, it may be that only nigral neurons degenerate because they express lower levels of vesicular uptake. Thus, although these results identify a component of resistance to the toxin using expression in a fibroblast, its reduced activity in midbrain neurons relative to the adrenal gland (and perhaps sympathetic ganglia) may account for the selective vulnerability of nigral cells. Chromaffin granule amine content and uptake activity vastly exceed that observed in synaptic vesicles from the central nervous system (Johnson, *Physiol. Rev.* 68:232–307 (1988)), suggesting that such differential expression exists and may well account for the observed differential susceptibility to MPP⁺.

Because Parkinson's disease usually spares chromaffin cells of the adrenal medulla and postganglionic sympathetic

neurons, differential expression of the vesicular amine transport protein has relevance for this idiopathic disorder. Because dopamine may induce oxidative stress (Cohen, *J. Neural Transmission Suppl.* 32:229–238 (1990)), an imbalance between membrane and vesicular catecholamine transport would lead to high cytoplasmic levels of dopamine as well as MPP⁺, and could be responsible for oxidative stress unrelated to an exogenous toxin (FIG. 10). FIG. 10 shows a presynaptic neuron above with mitochondrion, synaptic vesicles, equal plasma membrane uptake but less (left) and more (right) vesicular uptake. The concentration of toxin or neurotransmitter in the various compartments is indicated by the intensity of shading.

These results indicate the therapeutic potential for manipulation of the vesicle amine transport protein in PD. The transport protein presumably recognizes the same features of an exogenous or endogenous toxin that are recognized by the plasma membrane transporter, and which presumably account for the selective cell vulnerability observed in PD. However, in contrast to the plasma membrane transporter, increased expression of the vesicle transport protein has a protective effect. This approach to therapy has the advantage that it makes no assumptions about the exact nature of the toxin.

The above experiments demonstrate that the vesicular transport of neurotransmitter can be expressed in a cell without synaptic vesicles. Vesicular transport activity does not appear to require other specific synaptic vesicle components for functional expression. It requires only the activity of a more widely distributed vesicular H⁺-ATPase to generate the proton gradient that drives transport. The method of loading transfected non-neuronal cells with exogenous neurotransmitter followed by direct visualization of its cellular location as described herein provides a method to detect the intracellular transport activity in the absence of a high-affinity plasma membrane transporter.

As described below, the MPP⁺-resistant CHO transfectant provided a source for the isolation of sequences encoding a vesicle membrane protein having amine transport activity.

EXAMPLE 2

Cloning and Sequencing of Chromaffin Granule Amine Transporter Gene

This example identifies a cDNA clone that encodes the chromaffin granule amine transport protein.

Identification of cDNA clone encoding resistance to MPP⁺

To identify the cDNA sequences responsible for conferring resistance to MPP⁺, integrated plasmids were rescued from the primary CHO transformant obtained as described above in Example 1. A size-selected oligo-dT-primed PC12 cDNA library in the plasmid expression vector CDM8 was transfected into CHO cells, the stable transformants were selected in 1 mM MPP⁺ and the resistance of selected cells was found to be reversible with reserpine as described above in Example 1. The primary MPP⁺-resistant CHO transformant was amplified in culture and following cell lysis in 5M guanidinium isothiocyanate, the high molecular weight DNA prepared by repeated precipitation in isopropanol. After digestion with Not I, which cleaves the CDM8 vector once downstream of the cDNA insertion site as well as at rare eight-nucleotide recognition sites elsewhere in the genome, the DNA was religated and transformed into *E. coli* by electroporation as described by Stern-Bach et al., *J. Biol. Chem.* 265:3961–3966 (1990). Four pools of the derived plasmids containing approximately 50 colonies each were transfected into CHO cells and after two days were selected in 1 mM MPP⁺. Four weeks later, one pool gave rise to

several MPP⁺-resistant colonies, two pools gave rise to 1–2 colonies and the fourth pool and CDM8 vector gave rise to no healthy, resistant colonies. Restriction enzyme analysis of the plasmids in the pool conferring the highest frequency of resistance to MPP⁺ indicated three independent clones of 1.0, 1.3 and 2.5 kb. These plasmids were then transfected individually into CHO cells, which were again selected in 1 mM MPP⁺. After 2½ weeks, only cells transfected with the 2.5 kb cDNA (*mpp^{res}*) contained healthy CHO cells growing at a normal rate.

For Northern analysis, 10 µg total RNA was separated by electrophoresis through 2.2M formaldehyde/1.5% agarose, blotted to nylon (Hybond, Amersham), pre-hybridized in 50% formamide/5× SSC/5× Denhardt's solution/0.5% SDS/200 µg/ml salmon sperm DNA for 4 hours at 42° C., hybridized in the same solution with the *mpp^{res}* insert labelled by random priming at 42° C. for 16 hours, washed twice in 2× SSC/0.1% SDS for 30 minutes at room temperature, in 1× SSC/0.1% SDS for 1 hour at 50° C., 0.1× SSC/0.1% SDS for 1 hour at 50° C. and submitted to autoradiography with an intensifying screen for 18 hours. FIG. 11 shows the results of selection. The positions of 18S and 28S RNA are shown to the right in FIG. 11.

Dopamine-loaded fluorescence was carried out as described in Example 1. In order to examine vesicular amine transport directly in the resistant cells, cells were loaded with high concentrations of exogenous dopamine that circumvents the absence of a high affinity plasma membrane catecholamine transporter. Glyoxylic-acid-induced fluorescence was used to determine the intracellular distribution as described above in Example 1. The procedures were the same as described in Example 1 however a pure cDNA clone encoding the vesicle membrane transport protein, selected in the presence of MPP⁺, obtained as described herein, was used rather than the primary transformant.

As shown in FIG. 12, wild-type CHO cells showed diffuse cytoplasmic staining (FIG. 12a), while MPP⁺-resistant CHO cells (FIG. 12b) showed strong perinuclear and particulate cytoplasmic staining that was inhibited by 1 µM reserpine (FIG. 12c). This supports the hypothesis that vesicular amine transport confers resistance to MPP⁺.

From the pools of plasmid DNA, a single clone, designated *mpp^{res}*, was identified that conferred resistance to MPP⁺ after 2–3 weeks of selection in the toxin (FIG. 13) as noted above. FIG. 13 shows selection of CHO cells after transfection with *mpp^{res}* (left) and CDM8 vector alone (right). Only cells transfected with the *mpp^{res}* cDNA gave rise to healthy colonies, whereas cells transfected with other rescued cDNAs and the vector alone either died and detached or remained adherent, but with the refractile cytoplasmic inclusions characteristic of MPP⁺ toxicity. The persistently low frequency and late appearance of resistant colonies suggested that an additional factor might be required to express this mechanism of resistance. However, FIG. 11 shows that whereas wild-type CHO cells express none of these sequences, the resistant cells express over twenty times more than the PC12 cells serving as the source of the cDNA library. Thus, the low frequency of resistant colonies presumably results from the extraordinarily high levels of *mpp^{res}* expression which are required to confer drug resistance.

To address the possibility that selection in MPP⁺ may be required to express functional vesicular amine transport, the *mpp^{res}* cDNA was co-transfected with the selectable marker RSV-neo. Stable transformants were first selected in the neomycin analogue G418 (400 µg/ml) effective dose for one week and then in 1 mM MPP⁺ for two weeks. At the end of

selection, the residual cells were stained with 0.05% Coomassie Blue-R/10% acetic acid/50% methanol. As determined by dopamine-loaded fluorescence, selection in the neomycin analogue G418 yielded a high proportion of stable transformants that expressed easily detectable vesicular transport activity qualitatively similar to that observed after selection in MPP⁺ (FIG. 14, top). Thus, the expression of *mpp^{res}* alone suffices to confer vesicular amine transport, even in a non-neuronal cell.

The effects of various pharmacologic agents were tested to determine whether the properties of the protein encoded by the *mpp^{res}* cDNA corresponded with those expected for the chromaffin granule amine transport protein. PC12 dopamine uptake was determined in a 24-well plate pre-coated with poly-L-lysine. The cells were pre-incubated in Krebs-Ringer buffer containing 1 µM pargyline and the dose of the drug indicated for 15 minutes. 25 nM [³H]-dopamine was then added in the same buffer and after incubation at 37° C. for one hour, the cells were washed in cold Krebs-Ringer buffer, lysed and counted in Ecolite (ICN) as described by Greene and Rein, *Brain Res.* 129:247–263 (1978). Values were determined from three separate wells and the mean was expressed as percent of control uptake ± standard deviation. The dopamine fluorescence assay was carried out as described in Example 1, with an abbreviated 12 hour period of dopamine loading to minimize drug degradation. The dose required to inhibit maximal uptake by 50%, K_i, was estimated from the fluorescence data using a range of concentrations for each agent. The results are shown in Table 1.

TABLE 1

Agent	Dopamine Fluorescence	
	[³ H]-Dopamine Uptake (PC12)	(<i>mpp^{res}</i> CHO) K _i (est)
—	100 ± 5%	++++
reserpine 1 µM	23 ± 2	0 25 nM
tetrabenazine 100 µM	45 ± 2	++ 100 µM
verapamil 100 µM	21 ± 1	0 50 µM
desipramine 1 µM	30 ± 2	++++ 500 µM
chloroquine 500 µM	17 ± 1	0
NH ₄ Cl, 10 mM	51 ± 3	++

The ability of 50 nM reserpine to inhibit vesicular dopamine uptake completely in the fluorescence assay supports the identity of the cloned sequences as the chromaffin granule amine transport protein. Because vesicular transport is known to rely on a proton gradient, the effects of agents that disrupt vesicular acidification were also tested. As shown in Table 1, chloroquine inhibited vesicular amine uptake, but ammonium chloride inhibited uptake only partially, possibly due to expression of the transport protein in a protected compartment. Tetrabenazine and verapamil also inhibited transport, but at high concentrations that may reflect poor access in an intact cell as opposed to the chromaffin granule preparations in which transport has usually been characterized. It is also possible that the reduced sensitivity of the adrenal transporter to tetrabenazine may explain the known greater potency of that drug to deplete central rather than adrenal amine stores (Carlsson, *Hdbk. Exp. Pharmacol.* 19:529–92 (1965)). The pattern of drug response observed using the dopamine-loaded fluorescence assay in the CHO transfectant corresponded to the pattern observed using the uptake of labelled transmitter in intact PC12 cells (Table 1), indicating that the cloned cDNA conferred virtually all of the pharmacologic properties of the native vesicle amine transport protein.

The quantitative transport test was performed as described above on wild-type CHO cells and *mpp^{res}* CHO cells. The results are shown in FIG. 15. FIG. 15A shows the kinetics of incorporation for both wild-type and *mpp^{res}* cells. The incorporation by *mpp^{res}* cells was blocked by the proton ionophore carbonyl cyanide *m*-chlorophenylhydrazine (CCCP) at a concentration of 5 μ M. FIG. 15B shows a Lineweaver-Burke plot of dopamine transport by the *mpp^{res}* CHO cells based on a 2-minute incubation. The K_m was 2.3 μ M. FIG. 15C shows a dose-response analysis of the inhibition of dopamine transport by reserpine, tetrabenazine, and cocaine, based on a 2-minute incubation. These results show that the cloned and expressed CGAT gene yields the pharmacologic properties expected for chromaffin granule transport, with inhibition by both reserpine and tetrabenazine. The activity differs from plasma membrane amine transporters in showing no dependence on external Na^+ and no inhibition by cocaine. Thus, functional analysis of the activity encoded by the rescued cDNA shows that it confers vesicular amine transport.

Using the quantitative assay, the affinity for different substrates was also determined. The rank order for affinity was serotonin>epinephrine>dopamine>norepinephrine.

With respect to tissue distribution, a 3.0 kb RNA transcript for the cloned transport protein has been detected only in the rat adrenal gland. However, the level of expression was fairly low even in this tissue, where chromaffin cells constitute a large proportion of the cells, and it may be difficult to detect mRNA transcripts in tissues such as the midbrain where dopaminergic neurons constitute only a small fraction of all of the cells present. However, procedures for screening cDNA libraries from different tissues within species and for different species are available and may be screened as described above using a fragment of the cloned transport protein presented herein as a hybridization probe.

The insert from cDNA *mpp^{res}* was subcloned into pBlue-script™ (Stratagene, San Diego, Calif.) using standard procedures and sequenced on both strands by the dideoxy method using single-stranded templates and Sequenase (USB) as described by Sanger et al., *Proc. Natl. Acad. Sci. USA* 74:5463–5467 (1977). Alignment to the Gen Bank database (University of Wisconsin Genetics Program, Madison, Wis.) was performed at the UCLA biological computing facility (University of California, Los Angeles, Calif.) using profile-based methods as described by Gribskov et al., *Proc. Natl. Acad. Sci. USA* 84:4355–4358 (1987). The *MPP^{res}* cDNA conferring both resistance to *MPP⁺* and vesicular catecholamine transport contained a 2.5 kb insert. Sequence analysis of this insert showed that the first ATG occurred at the beginning of the largest open reading frame, in a context that conforms to the consensus for translation initiation (FIG. 1) (SEQ ID NO: 1) (Kozak, *Nucl. Acids. Res.* 12:857–872 (1984)). The predicted protein of 521 amino acids shows no strong homology to known proteins, and contains no apparent signal peptide, but does show extensive hydrophobic domains consistent with a membrane protein. The analysis of hydrophobic moment (Eisenberg et al., *J. Mol. Biol.* 179:125–142 (1984)) predicts twelve transmembrane domains, a structure characteristic of other known transport proteins. The largest hydrophilic loop occurs between membrane domains 1 and 2, and contains three potential sites for N-linked glycosylation.

The model in FIG. 16 shows the hydrophilic loop facing the lumen of the vesicle, with the other loops disposed accordingly, and both N- and C-termini in the cytoplasm. Previous biochemical studies of [^3H]-reserpine binding and

purification of the transport protein by functional reconstitution have suggested a molecular weight of 80 kd (Stern-Bach et al., supra), consistent with the sequence predicted from the clone *mpp^{res}*.

The cloned vesicle membrane amine transport protein identified herein shows no primary sequence similarity to Na^+ -dependent, plasma membrane neurotransmitter transport proteins, as expected from its distinct biological role, mechanism of action and pharmacology. Both types of neurotransmitter transporter are predicted to have twelve transmembrane domains, but the single large hydrophilic loop occurs between the first two transmembrane domains of the vesicle transport protein, and between the third and fourth transmembrane helices in the family of plasma membrane transporters (Guastella et al., *Science* 249:1303–1306 (1990); Pacholczyk et al., *Nature* 350:350–354 (1991); Shimada et al., *Science* 254:574–578 (1991); Kilty et al., *Science* 254:578–579 (1991) and Hoffman et al., *Science* 254:579–580 (1991)). However, it is striking that in both cases the loop resides on a topologically equivalent side of the membrane (i.e. inside the vesicle lumen and outside the cell), but the two classes of protein transport in opposing directions suggesting that this loop is not involved in recognition of the substances transported by the protein.

The cloned transport protein demonstrated weak but definite homology with a class of bacterial transporters including the tetracycline resistance genes from pBR322 (SEQ ID NO: 7) and Tn10 (SEQ ID NO: 8), the bacterial multi-drug resistance (BMR) transporter (SEQ ID NO: 9), and more remotely, with a methylenomycin resistance gene (SEQ ID NO: 6) (FIG. 17). The alignment occurred almost exclusively in the N-terminal half of these transporters, with conserved residues in both transmembrane helices and intermembrane loops, but not in the cytoplasmic N-terminus or large luminal loop between transmembrane domains 1 and 2. The structural similarity is further supported by functional homology between the proteins. First, both classes of transporter mediate the efflux of toxic compounds from the cell interior. Second, both act as proton exchangers. Third, both the chromaffin granule transporter and the bacterial BMR transporter have relatively low substrate specificity. Fourth, reserpine inhibits both the vesicular amine transport protein and the bacterial BMR transporter.

The above results demonstrate that the cloned sequence for the chromaffin vesicle membrane transport protein encodes a member of a novel class of mammalian proteins which transport neurotransmitters and other compounds into vesicles. The selection strategy used to isolate the clone also suggests a role for these proteins in treating Parkinson's disease. For example, expression of transport protein could be increased by gene transfer with herpes virus vectors or by using drugs that affect expression in animal models or tissue culture. It may also be that development of Parkinson's requires a genetic predisposition. Although the disease does not tend to run in families, twin studies have suggested a genetic component which may be required but is not sufficient to cause the disease (see Johnson et al., *Movement Disorders* 5:187–194 (1990)). Therefore, the cloned transporter protein of the invention may be used to identify mutations that predispose an individual to Parkinson's disease. With early diagnosis, it may be possible to prevent the disease by administering other drugs such as deprenyl which have been shown to slow the rate of progression of the disease.

EXAMPLE 3

Cloning and Sequencing of Synaptic Vesicle Amine Transporter Gene from Rat Brainstem

To address the possibility that aminergic populations of central nervous system cells express a synaptic vesicle amine transporter (SVAT) that is distinct from the chromaffin granule amine transporter (CGAT), a search for sequences related to CGAT but expressed in the brain was undertaken. Approximately 10^6 plaques from a bacteriophage λ gt10 rat brainstem library (Clontech, Palo Alto, Calif.) were screened on duplicate nylon filters (Biotrans, ICN, Costa Mesa, Calif.) with the CGAT probe labeled by random priming (Feinberg & Vogelstein, *Anal. Biochem.* 132:6-13 (1983)). The aqueous hybridization procedure of Boulton et al., supra, was used at 60° C., with washes at 55° C. Positive plaques were purified through two additional rounds of screening, subcloned into pBluescript (Stratagene, San Diego, Calif.), and sequenced by the chain termination method (Sanger & Coulson, supra).

The resulting DNA sequences provided the sequence of most of the SVAT gene. However, some gaps remained. To fill these gaps, PCR cloning was used to develop additional clones for sequencing (Marchuk et al., *Nucl. Acids Res.* 19:1154 (1991)).

PCR was performed by the following procedure: In a total volume of 50 μ l, the reaction mixture contained 15 μ g of library DNA, 2 mM $MgCl_2$, 2 mM each of the four deoxyribonucleoside triphosphates, 2.5 units of Tag polymerase (Perkin-Elmer-Cetus, Norwalk, Conn.), 1 \times PCR buffer (Perkin-Elmer-Cetus), and 100 pmole each of 2 primers: a library-specific primer, 5'-GATGATGGAGACCATGTGTTC-3' and a λ gt10-specific primer, 5'-GAAAGCTTCTTATGAGTATTTCTTCAAGGGTA-3'. The first cycle of PCR was 94° C. for 5 minutes for denaturing, 60° C. for 30 seconds for annealing, and 72° for 4 minutes for extension. Subsequently, 30 additional cycles were performed at 94° C. for 1 minute for denaturing, 60° C. for 30 seconds for annealing, and 72° for 4 minutes for extension. A final PCR cycle was then performed at 94° C. for 1 minute for denaturing, 60° C. for 30 seconds for annealing, and 72° for 10 minutes for extension.

The resulting PCR products were electrophoresed on a 1% low melting point agarose gel and DNA ranging in size from 4.3 kb to 5 kb was cut out of the gel. This DNA was then ligated at 14° C. with 200 ng of T-vector (pBluescript KSII⁺ cut with EcoRV). The ligated DNA was then incubated with Tag polymerase at 1 unit of polymerase per μ g plasmid in a 20- μ l reaction volume with 2 mM dTTP for 2 hours at 68° C. The resulting DNA was then extracted with phenol-chloroform and precipitated with ethanol.

The precipitated DNA was then used for transformation in *E. coli* and colonies were screened by hybridization using the original cDNA as a probe. In this way, additional clones were isolated that allowed the complete sequence of the SVAT gene to be determined. The DNA sequence of this gene and the amino acid sequence of the corresponding protein are shown in FIG. 2 (SEQ ID NO: 3, & 4). The protein is closely related in sequence to CGAT protein, and also has twelve transmembrane domains.

The relationship between the amino acid sequences of the two proteins is shown in FIG. 4. Extensive sequence divergence occurs principally in the large luminal loop located between the first two transmembrane proteins of both proteins, and to a lesser extent at the N- and C-termini.

Northern blotting showed that RNA capable of hybridizing to the cloned SVAT cDNA occurred as a transcript of approximately 4 kb in the midbrain, pons, or medulla, but not in the adrenal gland or other peripheral tissues. The RNA

used was isolated by disruption of the tissue in 6M guanidinium chloride followed by centrifugation through cesium chloride (Chirgwin et al., *Biochemistry* 18:5294-5299 (1979)). PolyA⁺ RNA was isolated by chromatography over oligo-dT cellulose (Aviv & Leder, supra). For Northern analysis, 10 μ g total or polyA⁺ RNA was separated by electrophoresis through 2.2M formaldehyde/1.5% agarose and blotted to nylon (Hybond, Amersham, Arlington Heights, Ill.). For high stringency aqueous hybridization, the hybridization procedure of Boulton et al., supra, was used at 68° C. The results are shown in FIG. 18B; Northern blots to CGAT cDNA are shown in FIG. 18A. The Northern blots show that RNA hybridizing to SVAT cDNA is found only in central nervous system tissues, and not in the adrenal gland or other tissues. These results support the existence of tissue-specific subtypes of the amine transport protein.

Further confirmation of the existence and expression of tissue-specific subtypes was obtained through in situ hybridization. The procedure for in situ hybridization is described in detail in Sternini et al., *Gastroenterology* 97:348-356 (1989). In brief, the animals were anesthetized with nembutal and perfused with 4% paraformaldehyde (PFA) in PBS. The brain was then dissected, postfixed for an additional 2 hours, cryoprotected with 25% sucrose in 4% PFA/PBS and section in a transverse plane at 30 μ m. The sections were then washed in 0.75 mg/ml glycine, digested in 1 μ g/ml proteinase K, 50 mM Tris-HCl, pH 8, 5 mM EDTA for 30 minutes at 37° C. and then treated with 0.25% acetic anhydride, 0.1M triethanolamine, pH 8 for 10 minutes at room temperature. Strand-specific RNA probes were prepared from the SVAT cDNA subcloned into pBluescript (Stratagene) using T7 RNA polymerase (Promega, Madison, Wis.) (Cox et al., *Dev. Biol.* 101:485-502 (1984)). After prehybridization in 50% formamide, 0.75M NaCl, 25 mM EDTA, 25 mM PIPES, pH 6.8, 1 \times Denhardt's solution, 0.2% SDS, 25 mM dithiothreitol, 250 μ g/ml denatured salmon sperm DNA, 250 μ g poly rA for more than one hour at 37° C., the sections were hybridized overnight at 55° C. in the same solution containing 5% dextran sulfate and 0.1 ng/ml of the labeled probe. The sections were washed in 4 \times standard saline citrate (SSC), 50 mM β -mercaptoethanol, treated with 50 μ g/ml RNase A for 30 minutes at 37° C., washed in 2 \times SSC, then in 0.1 \times SSC at 65° C. and finally in 0.1 \times SSC at room temperature overnight. The sections were mounted on gelatin-coated slides and exposed to autoradiographic film (KodakTM, Eastman Kodak, Rochester, N.Y.) for 4 days.

The results of in situ hybridization are shown in FIG. 19. Hybridizing cell populations appeared in the substantia nigra (SN), ventral tegmental area (VTA), locus coeruleus (LC), nucleus raphe pallidus (nrp), nucleus tractus solitarius (nts), and regions A1 and A5, but do not appear in adjacent areas. This is the location of dopaminergic, noradrenergic, and serotonergic cell populations expected to express such a synaptic vesicle amine transport protein.

The existence of different, through related, transporter proteins, expressed in the adrenal gland and in the brain raises the possibility of developing inhibitors or activators that differentially affect the two tissue-specific subtypes. Such inhibitors or activators would allow, for example, the development of compounds that affect adrenal gland activity without affecting the nervous system, or, conversely, compounds intended to treat disorders of the nervous system without affecting adrenal gland function.

EXAMPLE 4

Identification and Sequencing of Human Genomic DNA Sequence Hybridizing with Rat SVAT cDNA Probe

Because of expression of rat SVAT protein in aminergic central nervous system cells, an attempt was made to identify a corresponding human gene.

A human genomic library in phage λ EMBL 3 (Stratagene), obtained from Dr. J. Nathan, Johns Hopkins University, was first screened by Southern blotting with the rat CGAT cDNA probe at low stringency (Boulton et al., supra). The rat CGAT cDNA was radiolabeled by random priming (Feinberg & Vogelstein, *Anal. Biochem.* 132:6-13 (1983)). Hybridization was carried out for 16-24 hours at 60° C. in a hybridization solution containing 0.5M sodium phosphate, pH 7.0, 7% SDS, 1% BSA, and 1 mM EDTA. After hybridization, the filters were washed in 40 mM sodium phosphate, pH 7.2, 0.5% BSA, 5% SDS, 1 mM EDTA twice for one hour each at 50° C. and then in 40 mM sodium phosphate, pH 7.2, 1% SDS, 1 mM EDTA twice for one hour each at the same temperature. Positive plaques were picked after autoradiography with enhancement for five to seven days and purified through two additional rounds of screening.

To distinguish between the phage clones encoding CGAT (VAT1) and SVAT (VAT2), a radiolabeled Nco I fragment (+71 to +733) containing the divergent loop between the first and second predicted transmembrane domains was used to probe a Southern blot of DNA from the various phage clones. The hybridization and washes were carried out as described above but at 68° C. for high stringency and the 1.3 kb Eco RI fragment identified from one phage isolate was subcloned into pBS (Stratagene). Sequence analysis was carried out by the dideoxy chain-termination method of Sanger et al., *Proc. Natl. Acad. Sci. USA* 74:5463-5467 (1977). The sequence analysis of double-stranded plasmid DNA showed an exon with predicted amino acid sequence highly related to the large luminal loop in rat SVAT and unrelated to the same region of rat CGAT (FIG. 5) (SEQ ID NO: 5).

The sequence of the human genomic DNA corresponding to the rat SVAT DNA probe is shown in FIG. 5 (SEQ ID NO: 5). The sequenced region represents about 300 bases of an exon plus a few bases of an adjoining intron. The degree of homology between the exon and the rat SVAT cDNA is substantial, with stretches of up to 15 bases showing complete homology.

The sequence of the exon and surrounding introns in the SCAT (VAT2) genomic phage subclone were used to design the following oligonucleotide primers for PCR (from 5' to 3'): hloop 1, CTGACTAAAGTAGTCTGCC (SEQ ID NO: 14) (intron); hloop 2, TACAGAAATCCAGACGG (SEQ ID NO: 15) (exon); hloop 3, CGTCTGGATTCTGTAG (SEQ ID NO: 16) (exon); hloop 4, GGCATGGTGCTTTCTAG (SEQ ID NO: 16) (intron). The PCR was then performed by denaturing hloop 1 and 2 or hloop 3 and 4 with 25 ng genomic DNA in standard PCR buffer containing 1.5 mM MgCl₂ (Perkin-Elmer Cetus, Norwalk, Conn.) at 94° C. for four minutes, followed by 35 cycles of denaturation at 94° C. for one minute, annealing at 55° C. for two minutes, and extension at 72° C. for four minutes. After separation of the products by electrophoresis through 1.4% agarose, a Southern blot of the gel was hybridized to the radiolabeled 1.3 kb Eco RI subclone under high stringency and submitted to autoradiography.

The results are shown in FIG. 20. Despite the smaller size of the resulting fragment, amplification with hloop 1 and 2 oligonucleotide primers appears less efficient than with hloop 3 and 4, but both show the same pattern of amplification in the panel of cell hybrids. The pattern indicates localization of the human SVAT gene to chromosome 10.

For isolation of human SVAT cDNA, a human midbrain cDNA library in λ bacteriophage gt10 (Clontech, Palo Alto, Calif.) was screened with the rat SVAT cDNA as described above for the isolation of the human SVAT gene from the genomic library. Ten positive plaques were picked and purified and the phage DNA prepared. To determine which of the clones contained a full length cDNA, oligonucleotide primers from the 5' and 3' ends of rat SVAT were used to amplify the phage DNA by PCR as described above. A single clone yielded a PCR product of the appropriate size and the insert from this phage was subcloned for sequence analysis on both strands by the chain-termination method of Sanger et al., supra.

The use of the large luminal loop as a probe was to distinguish between the human equivalents of rat SVAT and rat CGAT, because this section of the DNA diverges extensively between the two genes. This probe should therefore selectively hybridize with the human SVAT gene rather than the human CGAT gene, assuming substantial homology between the rat and human sequences of the corresponding transporter genes. Sequence analysis of the subclone fragment confirmed a strong similarity to rat SVAT relative to rat CGAT in the region of the large luminal loop for the human gene. This sequence showed virtually no similarity to rat CGAT.

The sequence of the human SVAT cDNA is shown in FIG. 3 (SEQ ID NO: 12) and supports the identity of the isolated genomic phage clone as the human SVAT gene. In FIG. 21, a comparison of the predicted amino acid sequence encoded by the human SVAT DNA with the predicted amino acid sequences encoded by rat SVAT and CGAT cDNAs is shown. The predicted human SVAT protein sequence has 92.5% identity and 96.5% homology to rat SVAT. Most of the divergence occurs in the large luminal loop between the first two transmembrane domains, but the human and rat SVAT sequences still show considerable homology in this region, in contrast with the more striking divergence between rat CGAT and SVAT. A similar comparison of the cDNA sequences for human SVAT, rat SVAT, and rat CGAT is shown in FIG. 22.

The sequence of the human SVAT cDNA confirms the sequence of the exon obtained from the gene. In addition, human SVAT shows striking similarity to rat SVAT. The similarity includes both ends of the protein and the large luminal loop between transmembrane domains 1 and 2, a region of strong divergence between rat CGAT and rat SVAT. Conservation in these regions of the protein suggests an important physiologic role that may differ between SVAT and CGAT. A recent paper describes the isolation of a human SVAT cDNA with a somewhat different sequence than that presented here (C. K. Surratt et al., *FEBS Lett.* 318:325-330 (1993)). This different DNA sequence results in an amino acid sequence differing at 4 residues in the carboxy-terminal half of the protein. Although these differences may represent true species variation or a very closely related member of this gene family, the conservation of the residues reported here in two distinct genes from another species (rat SVAT and rat CGAT) strongly suggests that these amino acids do not vary within the human population for SVAT. Further, the substitution of a serine for a cysteine in the middle of transmembrane domain 7, a threonine for a lysine in the cytoplasmic loop following transmembrane domain 8, a proline for an alanine at the border of transmembrane domain 9, and an asparagine for an isoleucine in transmembrane domain 10, may well disrupt transporter structure or function. These changes cannot properly be considered conservative amino acid substitutions, because they alter

charge, polarity, and the tendency to form a α -helix. In addition to this clear sequence information obtained from both strands, the conservation of these critical residues in rat CGAT and rat SVAT strongly supports the sequence presented here.

EXAMPLE 5

Determination of Chromosomal Location of Human Gene Encoding the Chromaffin Granule Amine Transporter

In order to determine the chromosomal location of the human gene in coding the chromaffin granule amine transporter, we used Southern analysis of genomic DNA from mouse/human hybrid cell lines. A panel of 17 mouse/human somatic cell hybrids was derived from the fusion of

The results of Southern blot hybridization are shown in FIG. 23. Under conditions of high stringency, the rat CGAT cDNA as probed identified a unique set of Eco RI fragments in human cells, distinct from those present in mouse cells (FIG. 23). The number of fragments (3 with Eco RI digestion) suggested possible hybridization to several genes on different chromosomes. However, hybridization to a panel of DNA from mouse/human hybrids indicated the localization of all of these fragments to chromosome 8, with no discordant hybrids and no ambiguity (Table 2).

TABLE 2

	Human Chromosomes																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	
CGAT (VAT1)																									
<u>Concordant</u>																									
++		5	6	9	10	6	12	9	13	0	7	5	8	5	9	10	3	12	8	7	9	5	6	2	1
-/-		2	3	2	1	0	1	1	3	3	1	2	0	2	0	3	3	0	2	3	0	0	1	3	2
<u>Discordant</u>																									
+/-		8	7	4	3	7	1	4	0	13	6	8	5	8	4	3	10	1	5	6	4	8	7	11	12
-/+		1	0	1	2	3	2	2	0	0	2	1	3	1	3	0	0	3	1	0	3	3	2	0	1
Total discordant hybrids		9	7	5	5	10	3	6	0	13	8	9	8	9	7	3	10	4	6	6	7	11	9	11	13
Total informative hybrids		16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16
SVAT (VAT2)																									
<u>Concordant</u>																									
++		6	3	6	8	5	8	6	7	0	9	2	6	5	7	5	1	8	6	6	8	5	5	1	2
-/-		7	4	3	3	3	2	2	0	8	8	3	2	7	2	3	6	0	4	6	3	4	4	6	8
<u>Discordant</u>																									
+/-		3	6	3	1	4	1	1	2	9	0	7	3	4	2	4	8	1	3	3	1	4	4	8	7
-/+		1	4	5	5	5	6	6	8	0	0	5	6	1	6	5	2	8	4	2	5	4	4	2	0
Total discordant hybrids		4	10	8	6	9	7	7	10	9	0	12	9	5	8	9	10	9	7	5	6	8	8	10	7
Total Informative hybrids		17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17

thymidine kinase deficient mouse cells (B82, GM 0347 A) and normal male fibroblasts (IMR91) as described in T. Mohandas et al., *Somatic Cell & Mol. Genet.* 12:89-94 (1986). Cytogenetic analysis was carried out on a minimum of 30 Q-banded metaphases per hybrid clone.

To carry out Southern analysis, genomic DNA from the human and mouse parental cell lines and somatic cell hybrid clones was digested with the restriction endonuclease Eco RI (8 U/ μ g DNA). Approximately 10 μ g from each sample was electrophoresed through 1.2% agarose in 40 mM Tris-acetate, pH 7.4, 1 mM EDTA (TEA), transferred by capillary action to NytranTM (Schleicher & Schuell, Keene, N.H.) and immobilized by cross-linking with ultraviolet light. Prehybridization was performed at 0.5M sodium phosphate, pH 7.0, 7% SDS, 1% BSA, 1 mM EDTA for four hours at 60° C. and hybridization in the same solution containing the CGAT cDNA radiolabeled by random priming (Feinberg & Vogelstein, supra) for 24 hours at the same temperature (Boulton et al., supra). The filter was washed twice in 2 \times standard saline citrate (SSC), 0.1% SDS for 20 minutes each at 65° C., twice in 0.1 \times SSC, 0.1% SDS for 20 minutes at 65° C., and then exposed with an enhancing screen at -70° C. to XAR-5 X-ray film (Kodak, Rochester, N.Y.) for two to five days.

45

On the other hand, hybridization of a Southern blot from the same hybrids using the synaptic vesicle amine transporters cDNA as probe gave ambiguous results, perhaps as a result of cross-hybridization to other sequences in the human genomic DNA.

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In situ hybridization with a ³H-labeled cDNA probe confirmed the localization of the CGAT gene on human chromosome 8. For in situ hybridization, peripheral blood from normal donors was cultured for three days in RPMI 1640 supplemented with 20% fetal calf serum and 3% phytohemagglutinin, then synchronized with 0.1 μ M methotrexate overnight, washed twice in unsupplemented RPMI and incubated in 30 μ g/ml bromodeoxyuridine for seven hours. The cells were then arrested in metaphase with 0.5 μ g/ml colchicine, lysed in 0.075M KCl for fifteen minutes at 37° C., sedimented, fixed with Carnoy's mixture (methanol: acetic acid, 3:1), spread onto glass slides and dried.

60

For standard in situ hybridization with ³H-labeled probes, the purified insert from the CGAT cDNA was labeled by random priming with [³H]dNTPs to a specific activity of approximately 3 \times 10⁸ cpm/ μ g and the hybridization performed according to the method of Harper & Saunders,

65

Chromosoma 83:431-439 (1981) as modified by Cannizzaro & Emanuel, *Cytogenet. & Cell Genet.* 38:308-309 (1984). The slides were dipped in photographic emulsion, exposed for one week and all silver grains on or touching chromosomes were scored.

The results are shown in FIG. 24. The grain counts on nine chromosome spreads showed regional localization to 8p21.3 (Harnden & Klinger, "An International System for Human Cytogenetic Nomenclature," Karger, Basel (1985)).

Localization of human CGAT at 8p21.3 occurs relatively close to the genes for lipoprotein lipase at 8p22 (R. S. Sparkes et al., *Genomics* 1:138-144 (1987)), glutathione reductase at 8p21.1 (P. K. A. Jensen et al., *Ann. Genet.* 25:207-211 (1982)) and the light neurofilament chain at 8p21 (J. Hurst et al., *Cytogenet. Cell Genet.* 45: 30-31 (1987); M. J. Summerville et al., *Genome* 30: 499-500 (1988)). Deletions have similarly localized the genes for erythrocyte ankyrin (S. E. Lux et al., *Nature* 345: 736-739 (1990)), luteinizing hormone releasing hormone (T. L. Yang-Feng et al., *Cytogenet. Cell Genet.* 42: 7016 (1991)) and an alpha-one-like adrenergic receptor (D. A. Schwinn et al., *J. Biol. Chem.* 265:8183-8189 (1990); Yang-Feng et al. (1991), supra) to this general vicinity. Several disease loci occur in this region but none with a prominent autonomic phenotype to suggest a disturbance of monoamine synaptic transmission.

Hereditary spherocytosis usually affects red blood cells relatively selectively and does not show gross chromosomal abnormalities. However, recent reports describe hereditary spherocytosis due to a deletion of chromosome 8 from 8p11 to 8p21 (F. F. Costa et al., *New Engl. J. Med.* 323:1046-1050 (1990); Lux et al. (1990), supra). These patients have a more serious disorder affecting a wide range of systems in addition to the hematopoietic. The phenotype includes dysmorphic features, psychomotor retardation, and abnormal eye movements. Thus, the more serious phenotype presumably results from a monosomy for a variety of genes in addition to erythrocyte ankyrin that may include the gene for CGAT and so account for several of the associated abnormalities.

EXAMPLE 6

Determination of Chromosomal Location of Human Gene Encoding the Synaptic Vesicle Amine Transporter

The regional localization of SVAT in the human genome was determined by fluorescent in situ hybridization using a biotinylated probe derived from the genomic phage clone that contained the large lumenal loop of SVAT. The entire phage DNA was labeled by nick translation with biotinylated dATP (BioNick, Gibco/BRL, Gaithersburg, Md.), hybridized for 48 hours at 37° C. (Hybrisol VII, Oncor, Gaithersburg, Md.), washed in 50% formamide-2× SSC for two minutes at 37° C., in 2× SSC for two minutes at 37° C. and the hybridized probe visualized (Chromosome In Situ Kit, Oncor) with avidin-fluorescein under epifluorescence with a Leitz Orthoplan microscope after counterstaining

with propidium diiodide. To determine the specific region and to confirm the identification of the chromosome, the spreads were sequentially T-G banded (Cannizzaro & Emanuel (1984), supra).

5 The results of in situ hybridization are shown in FIG. 25. The twin florescent spots seen on 27 metaphase spreads confirm the presence of the human SVAT gene on chromosome 10 and indicated specific localization to the 10q25 region.

10 FIG. 26 shows the results of the analysis of sequentially T-G-banded chromosome spreads in order to verify both the chromosomal and regional localizations for both CGAT and SVAT. This indicates that the human CGAT gene is localized at chromosome 8p21.3, and the human SVAT gene is localized at chromosome 10q25. The latter result is based on the analysis of 19 chromosome spreads.

A number of previously characterized genes surround the human gene for SVAT on the long arm of chromosome 10. These include the genes from metabolic enzymes such as phosphoglycerate mutase, glutamate oxaloacetate transaminase (C. Junien et al., *Ann. Genet. (Paris)* 25:25-27 (1982)) and uroporphyrinogen III synthase (K. H. Astrin et al., *Hum. Genet.* 87:18-22 (1991)) as well as the gene for the insulin-degrading enzyme (J. A. Affholter et al., *Mol. Endocrinol.* 4:1125-1135 (1990)). Interestingly, the genes encoding two receptors from monoamines, the beta-1 adrenergic receptor and the alpha-1 adrenergic receptor (Yang-Feng et al. (1991), supra) also occur in this region. Although no inherited diseases with a simple behavioral phenotype show genetic linkage to this region, deletions from 10q25 and 10q26 to the end of the chromosome have appeared in the literature (R. C. J. Lewandowski et al., *Hum. Genet.* 42:339-343 (1978); R. F. Wegner et al., *Clin. Genet.* 19:130-133 (1981); M. Mulcahy et al., *Clin. Genet.* 21:33-35 (1982); G. Evans-Jones et al., *Clin. Genet.* 24:216-219 (1983)). The phenotype associated with this deletion as well as with a trisomy for the end of 10q (J. M. Klep-de-Pater et al., *Hum. Genet.* 46:29-40 (1979)) includes dysmorphic features and severe mental retardation, presumably resulting from a monosomy from multiple genes. SVAT almost certainly belongs to the genes lost in the deletion and so may have a significant role in the pathogenesis of the observed developmental delay and hypotonia. Monoamine cell populations project throughout the central nervous system and influence both cognitive processes and spinal reflexes.

As will be apparent to those skilled in the art to which the invention pertains, the present invention may be embodied in forms other than those specifically disclosed above without departing from the spirit or essential characteristics of the invention. The particular embodiments of the invention described above, are, therefore, to be considered as illustrative and not restrictive. The scope of the present invention is as set forth in the appended claims rather than being limited to the examples contained in the foregoing description.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 17

(2) INFORMATION FOR SEQ ID NO:1:

-continued

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2421 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v i) ORIGINAL SOURCE:

- (A) ORGANISM:
- Rattus rattus*

(i x) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 262..1824

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCTCGAGATC	CATTGTGCTC	TAAAGTCAGC	ACATCCACTT	TCAGAGAACA	GAGTCTCTGC	60										
TGTCTTGCCA	ACGGCTGCTC	CTTCCTCTCT	GAGTGCCTCA	CATCAAGATA	AGCTAGAAGT	120										
GAGCTTCACT	GGACCAGGCA	GACTTCTTCT	CCTATAAAGG	TGACAGAAGA	CCACATTTGT	180										
CGAGGGGTCT	TCCTAAGCCC	TGGGAGGAGA	AGCCCCCACC	ATCTCACTCC	CTACCCAGCC	240										
CAGCCTCCTG	CAGCCCTTGC	C	ATG	CTC	CAG	GTT	GTT	CTG	GGT	GCT	CCT	CAG	291			
			Met	Leu	Gln	Val	Val	Leu	Gly	Ala	Pro	Gln	10			
			1				5									
CGG	TTG	CTG	AAG	GAA	GGA	AGG	CAG	TCC	CGC	AAG	CTG	GTG	CTG	GTG	GTG	339
Arg	Leu	Leu	Lys	Glu	Gly	Arg	Gln	Ser	Arg	Lys	Leu	Val	Leu	Val	Val	25
			15				20									
GTG	TTC	GTG	GCT	CTG	CTT	CTG	GAC	AAC	ATG	CTG	CTC	ACT	GTG	GTG	GTG	387
Val	Phe	Val	Ala	Leu	Leu	Leu	Asp	Asn	Met	Leu	Leu	Thr	Val	Val	Val	40
			30				35									
CCC	ATT	GTG	CCC	ACC	TTC	CTG	TAC	GCG	ACA	GAG	TTC	AAA	GAC	AGC	AAC	435
Pro	Ile	Val	Pro	Thr	Phe	Leu	Tyr	Ala	Thr	Glu	Phe	Lys	Asp	Ser	Asn	55
			45				50									
TCT	TCT	CTG	CAT	AGG	GGT	CCT	TCT	GTA	AGC	TCC	CAG	CAA	GCT	CTC	ACC	483
Ser	Ser	Leu	His	Arg	Gly	Pro	Ser	Val	Ser	Ser	Gln	Gln	Ala	Leu	Thr	70
			60				65									
TCT	CCT	GCC	TTC	TCT	ACC	ATA	TTC	TCC	TTC	TTT	GAC	AAC	ACC	ACC	ACG	531
Ser	Pro	Ala	Phe	Ser	Thr	Ile	Phe	Ser	Phe	Phe	Asp	Asn	Thr	Thr	Thr	90
							80									
ACT	GTA	GAA	GAA	CAT	GTA	CCC	TTC	CGT	GTA	ACT	TGG	ACA	AAT	GGC	ACC	579
Thr	Val	Glu	Glu	His	Val	Pro	Phe	Arg	Val	Thr	Trp	Thr	Asn	Gly	Thr	105
							95									
ATC	CCT	CCT	CCA	GTC	ACT	GAA	GCC	AGC	TCA	GTA	CCA	AAA	AAC	AAC	TGC	627
Ile	Pro	Pro	Pro	Val	Thr	Glu	Ala	Ser	Ser	Val	Pro	Lys	Asn	Asn	Cys	120
							110									
TTG	CAA	GGG	ATA	GAG	TTC	TTA	GAA	GAA	GAA	AAC	GTT	CGG	ATT	GGG	ATT	675
Leu	Gln	Gly	Ile	Glu	Phe	Leu	Glu	Glu	Glu	Asn	Val	Arg	Ile	Gly	Ile	135
							125									
CTA	TTT	GCT	TCA	AAA	GCT	TTG	ATG	CAA	CTT	CTG	GTC	AAC	CCA	TTT	GTA	723
Leu	Phe	Ala	Ser	Lys	Ala	Leu	Met	Gln	Leu	Leu	Val	Asn	Pro	Phe	Val	150
							140									
GGA	CCT	CTT	ACT	AAC	AGG	ATT	GGC	TAT	CAC	ATC	CCC	ATG	TTT	GTT	GGC	771
Gly	Pro	Leu	Thr	Asn	Arg	Ile	Gly	Tyr	His	Ile	Pro	Met	Phe	Val	Gly	170
							155									
TTT	ATG	ATC	ATG	TTT	CTC	TCC	ACA	CTA	ATG	TTT	GCT	TTC	TCT	GGC	ACC	819
Phe	Met	Ile	Met	Phe	Leu	Ser	Thr	Leu	Met	Phe	Ala	Phe	Ser	Gly	Thr	185
							175									
TAT	GCC	CTG	CTA	TTT	GTG	GCC	CGA	ACT	CTC	CAA	GGC	ATT	GGA	TCT	TCG	867
Tyr	Ala	Leu	Leu	Phe	Val	Ala	Arg	Thr	Leu	Gln	Gly	Ile	Gly	Ser	Ser	

-continued

			190				195				200					
TTT	TCA	TCT	GTT	GCA	GGA	CTT	GGG	ATG	CTG	GCC	AGT	GTC	TAT	ACT	GAC	915
Phe	Ser	Ser	Val	Ala	Gly	Leu	Gly	Met	Leu	Ala	Ser	Val	Tyr	Thr	Asp	
			205				210				215					
AAC	TAT	GAG	AGA	GGG	AGA	GCC	ATG	GGA	ATT	GCT	TTG	GGG	GGC	CTG	GCC	963
Asn	Tyr	Glu	Arg	Gly	Arg	Ala	Met	Gly	Ile	Ala	Leu	Gly	Gly	Leu	Ala	
			220				225				230					
TTG	GGA	CTT	CTG	GTG	GGA	GCA	CCT	TTC	GGA	AGT	GTG	ATG	TAT	GAA	TTT	1011
Leu	Gly	Leu	Leu	Val	Gly	Ala	Pro	Phe	Gly	Ser	Val	Met	Tyr	Glu	Phe	
			235				240				245				250	
GTG	GGC	AAG	TCC	TCA	CCA	TTC	CTC	ATC	TTG	GCC	TTC	TTG	GCA	CTT	CTG	1059
Val	Gly	Lys	Ser	Ser	Pro	Phe	Leu	Ile	Leu	Ala	Phe	Leu	Ala	Leu	Leu	
			255				260				265					
GAT	GGA	GCT	CTC	CAA	CTT	TGC	ATC	CTA	TGG	CCT	TCG	AAA	GTG	TCT	CCT	1107
Asp	Gly	Ala	Leu	Gln	Leu	Cys	Ile	Leu	Trp	Pro	Ser	Lys	Val	Ser	Pro	
			270				275				280					
GAG	AGT	GCC	ATG	GGG	ACT	TCG	CTT	TTG	ACG	CTT	CTC	AAA	GAC	CCT	TAC	1155
Glu	Ser	Ala	Met	Gly	Thr	Ser	Leu	Leu	Thr	Leu	Leu	Lys	Asp	Pro	Tyr	
			285				290				295					
ATC	CTG	GTA	GCA	GCA	GGT	TCC	ATC	TGC	TTG	GCC	AAC	ATG	GGA	GTC	GCC	1203
Ile	Leu	Val	Ala	Ala	Gly	Ser	Ile	Cys	Leu	Ala	Asn	Met	Gly	Val	Ala	
			300				305				310					
ATA	CTA	GAG	CCC	ACG	CTG	CCC	ATC	TGG	ATG	ATG	CAG	ACC	ATG	TGC	TCC	1251
Ile	Leu	Glu	Pro	Thr	Leu	Pro	Ile	Trp	Met	Met	Gln	Thr	Met	Cys	Ser	
			315				320				325				330	
CCC	GAG	TGG	CAG	CTA	GGT	CTG	GCT	TTC	TTG	CCT	GCT	AGT	GTG	GCC	TAC	1299
Pro	Glu	Trp	Gln	Leu	Gly	Leu	Ala	Phe	Leu	Pro	Ala	Ser	Val	Ala	Tyr	
			335				340				345					
CTC	ATT	GGC	ACG	AAC	CTC	TTT	GGT	GTG	TTG	GCT	AAC	AAG	ATG	GGT	CGG	1347
Leu	Ile	Gly	Thr	Asn	Leu	Phe	Gly	Val	Leu	Ala	Asn	Lys	Met	Gly	Arg	
			350				355				360					
TGG	CTG	TGC	TCC	CTT	GTT	GGG	ATG	GTG	GCA	GTA	GGT	ATC	AGC	TTG	CTC	1395
Trp	Leu	Cys	Ser	Leu	Val	Gly	Met	Val	Ala	Val	Gly	Ile	Ser	Leu	Leu	
			365				370				375					
TGT	GTA	CCT	CTG	GCT	CAC	AAT	ATT	TTT	GGT	CTT	ATT	GGC	CCC	AAT	GCA	1443
Cys	Val	Pro	Leu	Ala	His	Asn	Ile	Phe	Gly	Leu	Ile	Gly	Pro	Asn	Ala	
			380				385				390					
GGC	CTT	GGC	TTT	GCC	ATA	GGA	ATG	GTG	GAT	TCC	TCT	CTG	ATG	CCC	ATC	1491
Gly	Leu	Gly	Phe	Ala	Ile	Gly	Met	Val	Asp	Ser	Ser	Leu	Met	Pro	Ile	
			395				400				405				410	
ATG	GGA	TAC	CTG	GTG	GAC	TTA	CGC	CAC	ACC	TCT	GTG	TAT	GGG	AGT	GTC	1539
Met	Gly	Tyr	Leu	Val	Asp	Leu	Arg	His	Thr	Ser	Val	Tyr	Gly	Ser	Val	
			415				420				425					
TAT	GCC	ATC	GCC	GAT	GTG	GCC	TTT	TGT	GTG	GGC	TTT	GCT	ATT	GGC	CCA	1587
Tyr	Ala	Ile	Ala	Asp	Val	Ala	Phe	Cys	Val	Gly	Phe	Ala	Ile	Gly	Pro	
			430				435				440					
TCT	ACT	GGG	GGT	GTT	ATC	GTA	CAG	GTC	ATT	GGC	TTT	CCT	TGG	CTC	ATG	1635
Ser	Thr	Gly	Gly	Val	Ile	Val	Gln	Val	Ile	Gly	Phe	Pro	Trp	Leu	Met	
			445				450				455					
GTC	ATC	ATT	GGT	ACC	ATC	AAC	ATC	ATT	TAT	GCT	CCT	CTC	TGC	TGC	TTC	1683
Val	Ile	Ile	Gly	Thr	Ile	Asn	Ile	Ile	Tyr	Ala	Pro	Leu	Cys	Cys	Phe	
			460				465				470					
CTG	CAG	AAC	CCG	CCA	GCT	AAG	GAG	GAG	AAG	CGT	GCA	ATT	CTG	AGC	CAG	1731
Leu	Gln	Asn	Pro	Pro	Ala	Lys	Glu	Glu	Lys	Arg	Ala	Ile	Leu	Ser	Gln	
			475				480				485				490	
GAA	TGC	CCC	ACA	GAG	ACC	CAG	ATG	TAC	ACA	TTC	CAG	AAG	CCC	ACA	AAG	1779
Glu	Cys	Pro	Thr	Glu	Thr	Gln	Met	Tyr	Thr	Phe	Gln	Lys	Pro	Thr	Lys	
			495				500				505					
GCG	TTT	CCA	CTA	GGA	GAG	AAC	AGC	GAT	GAT	CCT	AGC	AGC	GGG	GAG		1824
Ala	Phe	Pro	Leu	Gly	Glu	Asn	Ser	Asp	Asp	Pro	Ser	Ser	Gly	Glu		

-continued

510	515	520	
TAACTGCGGA	GGGCGATATC	TGAGCCTCAC	ATCTACAGGG ACCAGTCTAC TACAGATTCA 1884
ATAAATTTCA	CTTTCCTCTC	CTCCAGGCCA	CTGCCTTCCT CCCTTCTTAT TGATACCTTT 1944
CCTTTACTCA	CCTGTAAGTG	CAACCCACCA	CTCTCCCTCT GTGCTTTGAC ACCACCCATG 2004
GCCCACTTTT	TGTGGGAGGA	CAGTGCTATT	TCCTGCCAGG CCAAAGCGAA GCTGATTAAA 2064
GCTGAGTTGT	GACAAGTTCT	GCAAGGGGTG	ACTCACTTCC TGCAGGCAGG ACTGAACAAT 2124
GTGCCTGCGA	AATCAGGGGG	ACAAATGACA	AGCCTGCCTT TCTTCTCTGA TTGTTTTTTT 2184
TTTTTTTTTG	ACATATTACC	AATATGTCCT	AAAATTTGAC TTGTGTCCTG TGAAATGCTT 2244
TCCCCTTATT	TTTTCCAGTT	TAGCTTCTAT	ACATACGGGT TTTTGCTTAT TTTATGTGCT 2304
AAAATTGTTT	ACCTTCATTA	AGTGAGGCCT	TCCTACTTTC TTCATCGCCC AATTGAGAGG 2364
AAATAAACAA	CTTTCCTAGG	CTTGAAAAAA	AACTTTAGAG CACAATGGAT CTCGAGG 2421

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 521 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Leu	Gln	Val	Val	Leu	Gly	Ala	Pro	Gln	Arg	Leu	Leu	Lys	Glu	Gly	1	5	10	15
Arg	Gln	Ser	Arg	Lys	Leu	Val	Leu	Val	Val	Val	Phe	Val	Ala	Leu	Leu	20	25	30	
Leu	Asp	Asn	Met	Leu	Leu	Thr	Val	Val	Val	Pro	Ile	Val	Pro	Thr	Phe	35	40	45	
Leu	Tyr	Ala	Thr	Glu	Phe	Lys	Asp	Ser	Asn	Ser	Ser	Leu	His	Arg	Gly	50	55	60	
Pro	Ser	Val	Ser	Ser	Gln	Gln	Ala	Leu	Thr	Ser	Pro	Ala	Phe	Ser	Thr	65	70	75	80
Ile	Phe	Ser	Phe	Phe	Asp	Asn	Thr	Thr	Thr	Thr	Val	Glu	Glu	His	Val	85	90	95	
Pro	Phe	Arg	Val	Thr	Trp	Thr	Asn	Gly	Thr	Ile	Pro	Pro	Pro	Val	Thr	100	105	110	
Glu	Ala	Ser	Ser	Val	Pro	Lys	Asn	Asn	Cys	Leu	Gln	Gly	Ile	Glu	Phe	115	120	125	
Leu	Glu	Glu	Glu	Asn	Val	Arg	Ile	Gly	Ile	Leu	Phe	Ala	Ser	Lys	Ala	130	135	140	
Leu	Met	Gln	Leu	Leu	Val	Asn	Pro	Phe	Val	Gly	Pro	Leu	Thr	Asn	Arg	145	150	155	160
Ile	Gly	Tyr	His	Ile	Pro	Met	Phe	Val	Gly	Phe	Met	Ile	Met	Phe	Leu	165	170	175	
Ser	Thr	Leu	Met	Phe	Ala	Phe	Ser	Gly	Thr	Tyr	Ala	Leu	Leu	Phe	Val	180	185	190	
Ala	Arg	Thr	Leu	Gln	Gly	Ile	Gly	Ser	Ser	Phe	Ser	Ser	Val	Ala	Gly	195	200	205	
Leu	Gly	Met	Leu	Ala	Ser	Val	Tyr	Thr	Asp	Asn	Tyr	Glu	Arg	Gly	Arg	210	215	220	
Ala	Met	Gly	Ile	Ala	Leu	Gly	Gly	Leu	Ala	Leu	Gly	Leu	Leu	Val	Gly	225	230	235	240
Ala	Pro	Phe	Gly	Ser	Val	Met	Tyr	Glu	Phe	Val	Gly	Lys	Ser	Ser	Pro				

-continued

245				250				255							
Phe	Leu	Ile	Leu	Ala	Phe	Leu	Ala	Leu	Leu	Asp	Gly	Ala	Leu	Gln	Leu
			260					265					270		
Cys	Ile	Leu	Trp	Pro	Ser	Lys	Val	Ser	Pro	Glu	Ser	Ala	Met	Gly	Thr
		275					280					285			
Ser	Leu	Leu	Thr	Leu	Leu	Lys	Asp	Pro	Tyr	Ile	Leu	Val	Ala	Ala	Gly
	290					295					300				
Ser	Ile	Cys	Leu	Ala	Asn	Met	Gly	Val	Ala	Ile	Leu	Glu	Pro	Thr	Leu
305					310					315					320
Pro	Ile	Trp	Met	Met	Gln	Thr	Met	Cys	Ser	Pro	Glu	Trp	Gln	Leu	Gly
			325						330					335	
Leu	Ala	Phe	Leu	Pro	Ala	Ser	Val	Ala	Tyr	Leu	Ile	Gly	Thr	Asn	Leu
			340					345					350		
Phe	Gly	Val	Leu	Ala	Asn	Lys	Met	Gly	Arg	Trp	Leu	Cys	Ser	Leu	Val
		355					360					365			
Gly	Met	Val	Ala	Val	Gly	Ile	Ser	Leu	Leu	Cys	Val	Pro	Leu	Ala	His
	370					375					380				
Asn	Ile	Phe	Gly	Leu	Ile	Gly	Pro	Asn	Ala	Gly	Leu	Gly	Phe	Ala	Ile
385					390					395					400
Gly	Met	Val	Asp	Ser	Ser	Leu	Met	Pro	Ile	Met	Gly	Tyr	Leu	Val	Asp
			405						410					415	
Leu	Arg	His	Thr	Ser	Val	Tyr	Gly	Ser	Val	Tyr	Ala	Ile	Ala	Asp	Val
			420						425				430		
Ala	Phe	Cys	Val	Gly	Phe	Ala	Ile	Gly	Pro	Ser	Thr	Gly	Gly	Val	Ile
		435					440					445			
Val	Gln	Val	Ile	Gly	Phe	Pro	Trp	Leu	Met	Val	Ile	Ile	Gly	Thr	Ile
	450					455					460				
Asn	Ile	Ile	Tyr	Ala	Pro	Leu	Cys	Cys	Phe	Leu	Gln	Asn	Pro	Pro	Ala
465					470					475					480
Lys	Glu	Glu	Lys	Arg	Ala	Ile	Leu	Ser	Gln	Glu	Cys	Pro	Thr	Glu	Thr
			485						490					495	
Gln	Met	Tyr	Thr	Phe	Gln	Lys	Pro	Thr	Lys	Ala	Phe	Pro	Leu	Gly	Glu
			500						505				510		
Asn	Ser	Asp	Asp	Pro	Ser	Ser	Gly	Glu							
		515					520								

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1637 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v i) ORIGINAL SOURCE:

- (A) ORGANISM: Rattus rattus

(i x) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 72..1619

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGGCGCACGG ACAGAGACCC AGGCTGTGTG GCGCTATAAC CGCGCAGTCA CAGGCGAGCC 60
AGAGCAGAGC C ATG GCC CTG AGC GAT CTG GTG CTG CTG CGA TGG CTG CGG 110

-continued

	Met 1	Ala	Leu	Ser	Asp 5	Leu	Val	Leu	Leu	Arg 10	Trp	Leu	Arg			
GAC Asp	AGC Ser 15	CGC Arg	CAC His	TCG Ser	CGC Arg	AAA Lys 20	CTG Leu	ATC Ile	CTG Leu	TTC Phe	ATC Ile 25	GTG Val	TTC Phe	CTT Leu	GCG Ala	158
CTG Leu 30	CTG Leu	CTG Leu	GAC Asp	AAC Asn	ATG Met 35	CTG Leu	CTC Leu	ACC Thr	GTC Val	GTG Val	GTT Val 40	CCC Pro	ATC Ile	ATC Ile	CCC Pro 45	206
AGC Ser	TAT Tyr	CTG Leu	TAC Tyr	AGC Ser 50	ATT Ile	AAG Lys	CAT His	GAG Glu	AAA Lys 55	AAC Asn	TCT Ser	ACG Thr	GAA Glu	ATC Ile 60	CAG Gln	254
ACC Thr	ACC Thr	AGA Arg	CCA Pro 65	GAG Glu	CTC Leu	GTG Val	GTC Val	TCC Ser 70	ACC Thr	TCC Ser	GAA Glu	AGC Ser	ATC Ile 75	TTC Phe	TCT Ser	302
TAC Tyr	TAT Tyr	AAC Asn 80	AAC Asn	TCT Ser	ACT Thr	GTG Val	TTG Leu 85	ATC Ile	ACC Thr	GGG Gly	AAT Asn	GCC Ala 90	ACT Thr	GGG Gly	ACT Thr	350
CTT Leu 95	CCA Pro	GGA Gly	GGG Gly	CAG Gln	TCA Ser	CAC His 100	AAG Lys	GCT Ala	ACC Thr	AGC Ser	ACA Thr 105	CAG Gln	CAC His	ACT Thr	GTG Val	398
GCT Ala 110	AAC Asn	ACC Thr	ACT Thr	GTC Val	CCT Pro 115	TCG Ser	GAC Asp	TGT Cys	CCC Pro	AGT Ser 120	GAA Glu	GAC Asp	AGA Arg	GAC Asp	CTT Leu 125	446
CTG Leu	AAT Asn	GAG Glu	AAT Asn	GTG Val 130	CAA Gln	GTT Val	GGG Gly	CTG Leu	CTG Leu	TTT Phe 135	GCC Ala	TCC Ser	AAA Lys	GCC Ala 140	ACT Thr	494
GTC Val	CAG Gln	CTC Leu	CTC Leu 145	ACT Thr	AAC Asn	CCA Pro	TTC Phe	ATA Ile 150	GGA Gly	CTT Leu	CTG Leu	ACC Thr	AAC Asn 155	AGA Arg	ATT Ile	542
GGC Gly	TAT Tyr	CCA Pro 160	ATT Ile	CCC Pro	ATG Met	TTT Phe	GCC Ala 165	GGC Gly	TTC Phe	TGC Cys	ATC Ile	ATG Met 170	TTT Phe	ATC Ile	TCA Ser	590
ACA Thr 175	GTT Val	ATG Met	TTT Phe	GCC Ala	TTC Phe 180	TCC Ser	AGC Ser	AGC Ser	TAT Tyr	GCC Ala	TTC Phe 185	CTG Leu	CTG Leu	ATC Ile	GCC Ala	638
AGG Arg 190	TCC Ser	CTT Leu	CAG Gln	GGA Gly	ATT Ile 195	GGC Gly	TCC Ser	TCC Ser	TGC Cys	TCA Ser 200	TCC Ser	GTG Val	GCT Ala	GGG Gly	ATG Met 205	686
GGT Gly	ATG Met	CTG Leu	GCC Ala	AGC Ser 210	GTG Val	TAC Tyr	ACA Thr	GAT Asp	GAT Asp 215	GAG Glu	GAG Glu	AGG Arg	GGG Gly	AAG Lys 220	CCC Pro	734
ATG Met	GGC Gly	ATT Ile	GCT Ala 225	TTG Leu	GGT Gly	GGC Gly	CTG Leu	GCC Ala 230	ATG Met	GGA Gly	GTC Val	TTA Leu	GTG Val 235	GGA Gly	CCC Pro	782
CCC Pro	TTC Phe	GGG Gly 240	AGT Ser	GTG Val	CTC Leu	TAT Tyr	GAG Glu 245	TTT Phe	GTG Val	GGG Gly	AAG Lys	ACA Thr 250	GCT Ala	CCC Pro	TTC Phe	830
CTG Leu 255	GTG Val	CTA Leu	GCT Ala	GCC Ala	TTG Leu	GTG Val 260	CTC Leu	TTG Leu	GAT Asp	GGG Gly	GCT Ala 265	ATT Ile	CAG Gln	CTC Leu	TTT Phe	878
GTG Val 270	CTC Leu	CAG Gln	CCG Pro	TCC Ser	CGA Arg 275	GTA Val	CAG Gln	CCA Pro	GAG Glu	AGT Ser 280	CAG Gln	AAG Lys	GGG Gly	ACA Thr	CCT Pro 285	926
CTA Leu	ACG Thr	ACC Thr	TTG Leu	CTG Leu 290	AAG Lys	GAT Asp	CCA Pro	TAC Tyr	ATC Ile 295	CTC Leu	ATC Ile	GCT Ala	GCA Ala	GGC Gly 300	TCC Ser	974
ATC Ile	TGC Cys	TTT Phe	GCA Ala 305	AAC Asn	ATG Met	GGG Gly	ATA Ile 310	GCC Ala	ATG Met	CTG Leu	GAG Glu	CCC Pro	GCC Ala 315	CTG Leu	CCC Pro	1022

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ATC Ile	TGG Trp	ATG Met	ATG Met	GAG Glu	ACC Thr	ATG Met	TGT Cys	TCC Ser	CGA Arg	AAG Lys	TGG Trp	CAG Gln	CTG Leu	GGC Gly	GTT Val	1070
		320					325					330				
GCT Ala	TTC Phe	CTC Leu	CCG Pro	GCG Ala	AGC Ser	ATC Ile	TCT Ser	TAT Tyr	CTC Leu	ATT Ile	GGA Gly	ACC Thr	AAT Asn	ATT Ile	TTT Phe	1118
	335					340					345					
GGG Gly	ATA Ile	CTT Leu	GCA Ala	CAC His	AAA Lys	ATG Met	GGA Gly	AGG Arg	TGG Trp	CTA Leu	TGT Cys	GCT Ala	CTT Leu	CTG Leu	GGA Gly	1166
	350				355					360					365	
ATG Met	GTA Val	ATT Ile	GTT Val	GGA Gly	ATC Ile	AGC Ser	ATT Ile	TTA Leu	TGC Cys	ATC Ile	CCC Pro	TTT Phe	GCA Ala	AAA Lys	AAT Asn	1214
				370					375					380		
ATC Ile	TAT Tyr	GGA Gly	CTC Leu	ATC Ile	GCT Ala	CCC Pro	AAC Asn	TTT Phe	GGA Gly	GTT Val	GGT Gly	TTT Phe	GCA Ala	ATT Ile	GGG Gly	1262
			385					390					395			
ATG Met	GTG Val	GAC Asp	TCC Ser	TCT Ser	ATG Met	ATG Met	CCT Pro	ATC Ile	ATG Met	GGC Gly	TAC Tyr	CTG Leu	GTT Val	GAC Asp	CTG Leu	1310
		400					405					410				
CGG Arg	CAT His	GTG Val	TCT Ser	GTC Val	TAT Tyr	GGG Gly	AGT Ser	GTT Val	TAT Tyr	GCC Ala	ATT Ile	GCA Ala	GAC Asp	GTG Val	GCC Ala	1358
	415					420					425					
TTT Phe	TGT Cys	ATG Met	GGC Gly	TAT Tyr	GCT Ala	ATC Ile	GGT Gly	CCC Pro	TCT Ser	GCT Ala	GGT Gly	GGT Gly	GCC Ala	ATC Ile	GCA Ala	1406
	430				435			440		440					445	
AAG Lys	GCA Ala	ATT Ile	GGC Gly	TTT Phe	CCT Pro	TGG Trp	CTT Leu	ATG Met	ACA Thr	ATT Ile	ATT Ile	GGG Gly	ATA Ile	ATT Ile	GAT Asp	1454
				450					455					460		
ATC Ile	GCT Ala	TTT Phe	GCT Ala	CCA Pro	CTC Leu	TGC Cys	TTT Phe	TTC Phe	CTT Leu	CGA Arg	AGT Ser	CCA Pro	CCT Pro	GCT Ala	AAG Lys	1502
			465					470					475			
GAG Glu	GAA Glu	AAA Lys	ATG Met	GCT Ala	ATC Ile	CTC Leu	ATG Met	GAC Asp	CAC His	AAC Asn	TGT Cys	CCC Pro	ATT Ile	AAA Lys	AGA Arg	1550
		480					485					490				
AAG Lys	ATG Met	TAC Tyr	ACT Thr	CAG Gln	AAT Asn	AAT Asn	GTC Val	CAG Gln	TCA Ser	TAT Tyr	CCC Pro	ATC Ile	GGT Gly	GAT Asp	GAT Asp	1598
	495					500					505					
GAA Glu	GAA Glu	TCT Ser	GAA Glu	AGT Ser	GAC Asp	TGAGACCCTC TAACGTCGCC C									1637	
	510				515											

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 515 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Ala	Leu	Ser	Asp	Leu	Val	Leu	Leu	Arg	Trp	Leu	Arg	Asp	Ser	Arg
1				5					10					15	
His	Ser	Arg	Lys	Leu	Ile	Leu	Phe	Ile	Val	Phe	Leu	Ala	Leu	Leu	Leu
			20					25					30		
Asp	Asn	Met	Leu	Leu	Thr	Val	Val	Val	Pro	Ile	Ile	Pro	Ser	Tyr	Leu
		35					40					45			
Tyr	Ser	Ile	Lys	His	Glu	Lys	Asn	Ser	Thr	Glu	Ile	Gln	Thr	Thr	Arg
	50					55					60				
Pro	Glu	Leu	Val	Val	Ser	Thr	Ser	Glu	Ser	Ile	Phe	Ser	Tyr	Tyr	Asn
	65				70					75					80
Asn	Ser	Thr	Val	Leu	Ile	Thr	Gly	Asn	Ala	Thr	Gly	Thr	Leu	Pro	Gly

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85					90					95					
Gly	Gln	Ser	His	Lys	Ala	Thr	Ser	Thr	Gln	His	Thr	Val	Ala	Asn	Thr
			100					105					110		
Thr	Val	Pro	Ser	Asp	Cys	Pro	Ser	Glu	Asp	Arg	Asp	Leu	Leu	Asn	Glu
		115					120					125			
Asn	Val	Gln	Val	Gly	Leu	Leu	Phe	Ala	Ser	Lys	Ala	Thr	Val	Gln	Leu
	130					135					140				
Leu	Thr	Asn	Pro	Phe	Ile	Gly	Leu	Leu	Thr	Asn	Arg	Ile	Gly	Tyr	Pro
145					150					155					160
Ile	Pro	Met	Phe	Ala	Gly	Phe	Cys	Ile	Met	Phe	Ile	Ser	Thr	Val	Met
				165					170					175	
Phe	Ala	Phe	Ser	Ser	Ser	Tyr	Ala	Phe	Leu	Leu	Ile	Ala	Arg	Ser	Leu
			180					185					190		
Gln	Gly	Ile	Gly	Ser	Ser	Cys	Ser	Ser	Val	Ala	Gly	Met	Gly	Met	Leu
		195					200					205			
Ala	Ser	Val	Tyr	Thr	Asp	Asp	Glu	Glu	Arg	Gly	Lys	Pro	Met	Gly	Ile
	210					215					220				
Ala	Leu	Gly	Gly	Leu	Ala	Met	Gly	Val	Leu	Val	Gly	Pro	Pro	Phe	Gly
225					230					235					240
Ser	Val	Leu	Tyr	Glu	Phe	Val	Gly	Lys	Thr	Ala	Pro	Phe	Leu	Val	Leu
				245					250					255	
Ala	Ala	Leu	Val	Leu	Leu	Asp	Gly	Ala	Ile	Gln	Leu	Phe	Val	Leu	Gln
			260					265					270		
Pro	Ser	Arg	Val	Gln	Pro	Glu	Ser	Gln	Lys	Gly	Thr	Pro	Leu	Thr	Thr
		275					280					285			
Leu	Leu	Lys	Asp	Pro	Tyr	Ile	Leu	Ile	Ala	Ala	Gly	Ser	Ile	Cys	Phe
	290					295					300				
Ala	Asn	Met	Gly	Ile	Ala	Met	Leu	Glu	Pro	Ala	Leu	Pro	Ile	Trp	Met
305					310					315					320
Met	Glu	Thr	Met	Cys	Ser	Arg	Lys	Trp	Gln	Leu	Gly	Val	Ala	Phe	Leu
				325					330					335	
Pro	Ala	Ser	Ile	Ser	Tyr	Leu	Ile	Gly	Thr	Asn	Ile	Phe	Gly	Ile	Leu
			340					345					350		
Ala	His	Lys	Met	Gly	Arg	Trp	Leu	Cys	Ala	Leu	Leu	Gly	Met	Val	Ile
		355					360					365			
Val	Gly	Ile	Ser	Ile	Leu	Cys	Ile	Pro	Phe	Ala	Lys	Asn	Ile	Tyr	Gly
	370					375					380				
Leu	Ile	Ala	Pro	Asn	Phe	Gly	Val	Gly	Phe	Ala	Ile	Gly	Met	Val	Asp
385					390					395					400
Ser	Ser	Met	Met	Pro	Ile	Met	Gly	Tyr	Leu	Val	Asp	Leu	Arg	His	Val
				405					410					415	
Ser	Val	Tyr	Gly	Ser	Val	Tyr	Ala	Ile	Ala	Asp	Val	Ala	Phe	Cys	Met
			420					425					430		
Gly	Tyr	Ala	Ile	Gly	Pro	Ser	Ala	Gly	Gly	Ala	Ile	Ala	Lys	Ala	Ile
		435					440					445			
Gly	Phe	Pro	Trp	Leu	Met	Thr	Ile	Ile	Gly	Ile	Ile	Asp	Ile	Ala	Phe
	450					455					460				
Ala	Pro	Leu	Cys	Phe	Phe	Leu	Arg	Ser	Pro	Pro	Ala	Lys	Glu	Glu	Lys
465					470					475					480
Met	Ala	Ile	Leu	Met	Asp	His	Asn	Cys	Pro	Ile	Lys	Arg	Lys	Met	Tyr
				485					490					495	
Thr	Gln	Asn	Asn	Val	Gln	Ser	Tyr	Pro	Ile	Gly	Asp	Asp	Glu	Glu	Ser
			500					505					510		

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Glu Ser Asp
515

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 332 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
- (i i) MOLECULE TYPE: DNA (genomic)
- (i i i) HYPOTHETICAL: NO
- (i v) ANTI-SENSE: NO
- (v i) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
- (x i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

CAATGAGAAG AATGCTACAG AAATCCAGAC GGCCTGGCCA GTGCACACGG CCTCCATCTC      60
AGACAGCTTC CAGAGCATCT TCTCCTATTA TGATAACTCG ACTATGGTCA CCGGGAATGC      120
TACCAGAGAC CTGACACTTC ATCAGACCGC CACACAGCAC ATGGTGACCA AGGCCTGCCG      180
TCTTCCTTCC GACTGTCCCA GTGAAGACAA AGACCTCCTG AATGAAAAGC TGCAAGTTGG      240
TCTGTTGTTT GCCTCGAAAG CCACCGTCCA GTCATCACC AACCCITTCA TAGGACTACT      300
GACCAACAGG TAGGGCAGAC TACTTTAGTC AG                                     332

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(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 203 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (i i) MOLECULE TYPE: peptide
- (i i i) HYPOTHETICAL: NO
- (v) FRAGMENT TYPE: internal
- (v i) ORIGINAL SOURCE:
 (A) ORGANISM: Streptomyces plasmid
- (x i) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Thr Ala Glu Val Pro Ala Gly Gly Arg Arg Asp Val Pro Ser Gly Val
1          5          10
Lys Ile Thr Ala Leu Ala Thr Gly Phe Val Met Ala Thr Leu Asp Val
20        25        30
Thr Val Val Asn Val Ala Gly Ala Thr Ile Gln Glu Ser Leu Asp Thr
35        40        45
Thr Leu Thr Gln Leu Thr Trp Ile Val Asp Gly Tyr Val Leu Thr Phe
50        55        60
Ala Ser Leu Leu Met Leu Ala Gly Gly Leu Ala Asn Arg Ile Gly Ala
65        70        75        80
Lys Thr Val Tyr Leu Trp Gly Met Gly Val Phe Phe Leu Ala Ser Leu
85        90        95
Ala Cys Ala Leu Ala Pro Thr Ala Glu Thr Leu Ile Ala Ala Arg Leu
100       105       110
Val Gln Gly Ala Gly Ala Ala Leu Phe Met Pro Ser Ser Leu Ser Leu
115       120       125
Leu Val Phe Ser Phe Pro Glu Lys Arg Gln Arg Thr Arg Met Leu Gly
130       135       140

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Leu	Trp	Ser	Ala	Ile	Val	Ala	Thr	Ser	Ser	Gly	Leu	Gly	Pro	Thr	Val
145					150					155					160
Gly	Gly	Leu	Met	Val	Ser	Ala	Phe	Gly	Trp	Glu	Ser	Ile	Phe	Leu	Leu
				165					170					175	
Asn	Leu	Pro	Ile	Gly	Ala	Ile	Gly	Met	Ala	Met	Thr	Tyr	Arg	Tyr	Ile
			180					185					190		
Ala	Ala	Thr	Glu	Ser	Arg	Ala	Thr	Arg	Leu	Ala					
		195					200								

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 196 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(i i i) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(v i) ORIGINAL SOURCE:

(A) ORGANISM: Escherichia coli plasmid

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met	Lys	Ser	Asn	Asn	Ala	Leu	Ile	Val	Ile	Leu	Gly	Thr	Val	Thr	Leu
1				5					10					15	
Asp	Ala	Val	Gly	Ile	Gly	Leu	Val	Met	Pro	Val	Leu	Pro	Gly	Leu	Leu
			20					25					30		
Arg	Asp	Ile	Val	His	Ser	Asp	Ser	Ile	Ala	Ser	His	Tyr	Gly	Val	Leu
		35					40					45			
Leu	Ala	Leu	Tyr	Ala	Leu	Met	Gln	Phe	Leu	Cys	Ala	Pro	Val	Leu	Gly
	50					55				60					
Ala	Leu	Ser	Asp	Arg	Phe	Gly	Arg	Arg	Pro	Val	Leu	Leu	Ala	Ser	Leu
65				70					75						80
Leu	Gly	Ala	Thr	Ile	Asp	Tyr	Ala	Ile	Met	Ala	Thr	Thr	Pro	Val	Leu
				85				90						95	
Trp	Ile	Leu	Tyr	Ala	Gly	Arg	Ile	Val	Ala	Gly	Ile	Thr	Gly	Ala	Thr
			100					105					110		
Gly	Ala	Val	Ala	Gly	Ala	Tyr	Ile	Ala	Asp	Ile	Thr	Asp	Gly	Glu	Asp
		115					120					125			
Arg	Ala	Arg	His	Phe	Gly	Leu	Met	Ser	Ala	Cys	Phe	Gly	Val	Gly	Met
	130					135					140				
Val	Ala	Gly	Pro	Val	Ala	Gly	Gly	Leu	Leu	Gly	Ala	Ile	Ser	Leu	His
145					150					155					160
Ala	Pro	Phe	Leu	Ala	Ala	Ala	Val	Leu	Asn	Gly	Leu	Asn	Leu	Leu	Leu
				165					170					175	
Gly	Cys	Phe	Leu	Met	Gln	Glu	Ser	His	Lys	Gly	Glu	Arg	Arg	Pro	Met
			180					185					190		
Pro	Leu	Arg	Ala												
			195												

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 194 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

-continued

(i i i) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(v i) ORIGINAL SOURCE:

(A) ORGANISM: Transposon 10

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```

Met  Asn  Ser  Ser  Thr  Lys  Ile  Ala  Leu  Val  Ile  Thr  Leu  Leu  Asp  Ala
 1          5          10          15
Met  Gly  Ile  Gly  Leu  Ile  Met  Pro  Val  Leu  Pro  Thr  Leu  Leu  Arg  Glu
 20          25          30
Phe  Ile  Ala  Ser  Glu  Asp  Ile  Ala  Asn  His  Phe  Gly  Val  Leu  Leu  Ala
 35          40          45
Leu  Tyr  Ala  Leu  Met  Gln  Val  Ile  Phe  Ala  Pro  Trp  Leu  Gly  Lys  Met
 50          55          60
Ser  Asp  Arg  Phe  Gly  Arg  Arg  Pro  Val  Leu  Leu  Leu  Ser  Leu  Ile  Gly
 65          70          75          80
Ala  Ser  Leu  Asp  Tyr  Leu  Leu  Leu  Ala  Phe  Ser  Ser  Ala  Leu  Trp  Met
 85          90          95
Leu  Tyr  Leu  Gly  Arg  Leu  Leu  Ser  Gly  Ile  Thr  Gly  Ala  Thr  Gly  Ala
100          105          110
Val  Ala  Ala  Ser  Val  Ile  Ala  Asp  Thr  Thr  Ser  Ala  Ser  Gln  Arg  Val
115          120          125
Lys  Trp  Phe  Gly  Trp  Leu  Gly  Ala  Ser  Phe  Gly  Leu  Gly  Leu  Ile  Ala
130          135          140
Gly  Pro  Ile  Ile  Gly  Gly  Phe  Ala  Gly  Glu  Ile  Ser  Pro  His  Ser  Pro
145          150          155          160
Phe  Phe  Ile  Ala  Ala  Leu  Leu  Asn  Ile  Val  Thr  Phe  Leu  Val  Val  Met
165          170          175
Phe  Trp  Phe  Arg  Glu  Thr  Lys  Asn  Thr  Arg  Asp  Asn  Thr  Asp  Thr  Glu
180          185          190
Val  Gly

```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 195 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(i i i) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(v i) ORIGINAL SOURCE:

(A) ORGANISM: Bacillus subtilis plasmid

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```

Met  Glu  Lys  Lys  Asn  Ile  Thr  Leu  Thr  Ile  Leu  Leu  Thr  Asn  Leu  Phe
 1          5          10          15
Ile  Ala  Phe  Leu  Gly  Ile  Gly  Leu  Val  Ile  Pro  Val  Thr  Pro  Thr  Ile
 20          25          30
Met  Asn  Glu  Leu  His  Leu  Ser  Gly  Thr  Ala  Val  Gly  Tyr  Met  Val  Ala
 35          40          45
Cys  Phe  Ala  Ile  Thr  Gln  Leu  Ile  Val  Ser  Pro  Ile  Ala  Gly  Arg  Trp
 50          55          60
Val  Asp  Arg  Phe  Gly  Arg  Lys  Ile  Met  Ile  Val  Ile  Gly  Leu  Leu  Phe

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65					70					75					80					
Phe	Ser	Val	Ser	Glu	Phe	Leu	Phe	Gly	Ile	Gly	Lys	Thr	Val	Glu	Met					
				85					90					95						
Leu	Phe	Ile	Thr	Arg	Met	Leu	Gly	Gly	Ile	Ser	Ala	Pro	Phe	Ile	Met					
			100				105						110							
Pro	Gly	Val	Thr	Ala	Phe	Ile	Ala	Asp	Ile	Thr	Thr	Ile	Lys	Thr	Arg					
		115					120					125								
Pro	Lys	Ala	Leu	Gly	Tyr	Met	Ser	Ala	Ala	Ile	Ser	Thr	Gly	Phe	Ile					
	130					135					140									
Ile	Gly	Pro	Gly	Ile	Gly	Gly	Phe	Leu	Ala	Glu	Val	His	Ser	Arg	Leu					
145				150						155					160					
Pro	Phe	Phe	Phe	Ala	Ala	Ala	Phe	Ala	Leu	Leu	Ala	Ala	Ile	Leu	Ser					
				165					170					175						
Ile	Leu	Thr	Leu	Arg	Glu	Pro	Glu	Arg	Asn	Pro	Glu	Asn	Gln	Glu	Ile					
			180				185						190							
Lys	Gly	Gln																		
		195																		

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 220 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(i i i) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(v i) ORIGINAL SOURCE:

- (A) ORGANISM: Rattus rattus

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Arg	Leu	Leu	Lys	Glu	Gly	Arg	Gln	Ser	Arg	Lys	Leu	Val	Leu	Val	Val					
1				5					10					15						
Val	Phe	Val	Ala	Leu	Leu	Leu	Asp	Asn	Met	Leu	Leu	Thr	Val	Val	Val					
			20					25					30							
Pro	Ile	Val	Pro	Thr	Phe	Leu	Tyr	Ala	Thr	Glu	Phe	Lys	Asp	Ser	Asn					
		35					40					45								
Ser	Ser	Leu	His	Arg	Gly	Pro	Ser	Val	Ser	Ser	Gln	Glu	Glu	Asn	Val					
	50					55					60									
Arg	Ile	Gly	Ile	Leu	Phe	Ala	Ser	Lys	Ala	Leu	Met	Gln	Leu	Leu	Val					
65				70					75					80						
Asn	Pro	Phe	Val	Gly	Pro	Leu	Thr	Asn	Arg	Ile	Gly	Tyr	His	Ile	Pro					
				85					90					95						
Met	Phe	Val	Gly	Phe	Met	Ile	Met	Phe	Leu	Ser	Thr	Leu	Met	Phe	Ala					
			100					105					110							
Phe	Ser	Gly	Thr	Tyr	Ala	Leu	Leu	Phe	Val	Ala	Arg	Thr	Leu	Gln	Gly					
		115					120					125								
Ile	Gly	Ser	Ser	Phe	Ser	Ser	Val	Ala	Gly	Leu	Gly	Met	Leu	Ala	Ser					
	130					135					140									
Val	Tyr	Thr	Asp	Asn	Tyr	Glu	Arg	Gly	Arg	Ala	Met	Gly	Leu	Ala	Leu					
145				150						155					160					
Gly	Gly	Leu	Ala	Leu	Gly	Leu	Leu	Val	Gly	Ala	Pro	Phe	Gly	Ser	Val					
			165						170					175						
Met	Tyr	Glu	Phe	Val	Gly	Lys	Ser	Ser	Pro	Phe	Leu	Ile	Leu	Ala	Phe					
			180					185						190						

-continued

Leu	Ala	Leu	Leu	Asp	Gly	Ala	Leu	Gln	Leu	Cys	Ile	Leu	Trp	Pro	Ser
		195					200					205			
Lys	Val	Ser	Pro	Glu	Ser	Ala	Met	Gly	Thr	Ser	Leu				
	210					215					220				

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 220 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: peptide

(i i i) HYPOTHETICAL: YES

(v) FRAGMENT TYPE: internal

(v i) ORIGINAL SOURCE:

(A) ORGANISM: Consensus sequence

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Asn	Xaa	Xaa	Leu	Xaa	Val
1				5					10					15	
Xaa	Leu	Xaa	Xaa	Leu	Leu	Leu	Asp	Xaa	Met	Gly	Ile	Gly	Leu	Val	Val
			20					25					30		
Pro	Val	Xaa	Pro	Thr	Leu	Leu	Xaa	Glu	Xaa	Xaa	Xaa	Ser	Asp	Xaa	Xaa
		35					40					45			
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
	50					55						60			
Xaa	Xaa	Gly	Val	Leu	Leu	Ala	Xaa	Tyr	Ala	Leu	Met	Gln	Leu	Xaa	Xaa
65					70					75					80
Xaa	Pro	Xaa	Xaa	Gly	Xaa	Leu	Xaa	Asp	Arg	Phe	Gly	Arg	Xaa	Xaa	Val
				85					90					95	
Leu	Leu	Xaa	Gly	Leu	Leu	Xaa	Xaa	Xaa	Leu	Xaa	Xaa	Leu	Leu	Phe	Ala
			100					105						110	
Phe	Xaa	Xaa	Thr	Xaa	Xaa	Met	Leu	Xaa	Xaa	Xaa	Arg	Leu	Leu	Xaa	Gly
		115					120					125			
Ile	Xaa	Xaa	Ala	Phe	Xaa	Xaa	Xaa	Ala	Xaa	Xaa	Xaa	Xaa	Leu	Ile	Ala
	130					135						140			
Asp	Xaa	Thr	Asp	Xaa	Xaa	Xaa	Arg	Xaa	Arg	Xaa	Xaa	Gly	Xaa	Met	Ser
145					150					155					160
Ala	Xaa	Phe	Xaa	Xaa	Gly	Leu	Ile	Xaa	Gly	Pro	Xaa	Ile	Gly	Gly	Xaa
				165					170					175	
Xaa	Xaa	Glu	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Pro	Phe	Xaa	Xaa	Ala	Ala	Leu
			180					185					190		
Leu	Asn	Leu	Leu	Xaa	Xaa	Xaa	Leu	Xaa	Met	Phe	Xaa	Leu	Arg	Glu	Xaa
		195					200					205			
Xaa	Xaa	Xaa	Xaa	Glu	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa			
	210					215						220			

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1898 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i i i) HYPOTHETICAL: NO

-continued

(i v) ANTI-SENSE: NO

(v i) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(i x) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 34..1575

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGCGCAAGCG	ACCCCGAGCG	GAGCCCCGGA	GCC	ATG	GCC	CTG	AGC	GAG	CTG	GCG							54
				Met	Ala	Leu	Ser	Glu	Leu	Ala							
				1				5									
CTG	GTC	CGC	TGG	CTG	CAG	GAG	AGC	CGC	CGC	TCG	CGG	AAG	CTC	ATC	CTG		102
Leu	Val	Arg	Trp	Leu	Gln	Glu	Ser	Arg	Arg	Ser	Arg	Lys	Leu	Ile	Leu		
		10					15					20					
TTC	ATC	GTG	TTC	CTG	GCG	CTG	CTG	CTG	GAC	AAC	ATG	CTG	CTC	ACT	GTC		150
Phe	Ile	Val	Phe	Leu	Ala	Leu	Leu	Leu	Asp	Asn	Met	Leu	Leu	Thr	Val		
	25					30					35						
GTG	GTC	CCC	ATC	ATC	CCA	AGT	TAT	CTG	TAC	AGC	ATT	AAG	CAT	GAG	AAG		198
Val	Val	Pro	Ile	Ile	Pro	Ser	Tyr	Leu	Tyr	Ser	Ile	Lys	His	Glu	Lys		
	40				45					50					55		
AAT	GCT	ACA	GAA	ATC	CAG	ACG	GCC	AGG	CCA	GTG	CAC	ACT	GCC	TCC	ATC		246
Asn	Ala	Thr	Glu	Ile	Gln	Thr	Ala	Arg	Pro	Val	His	Thr	Ala	Ser	Ile		
				60					65						70		
TCA	GAC	AGC	TTC	CAG	AGC	ATC	TTC	TCC	TAT	TAT	GAT	AAC	TCG	ACT	ATG		294
Ser	Asp	Ser	Phe	Gln	Ser	Ile	Phe	Ser	Tyr	Tyr	Asp	Asn	Ser	Thr	Met		
			75					80						85			
GTC	ACC	GGG	AAT	GCT	ACC	AGA	GAC	CTG	ACA	CTT	CAT	CAG	ACC	GCC	ACA		342
Val	Thr	Gly	Asn	Ala	Thr	Arg	Asp	Leu	Thr	Leu	His	Gln	Thr	Ala	Thr		
		90					95					100					
CAG	CAC	ATG	GTG	ACC	AAC	GCG	TCC	GCT	GTT	CCT	TCC	GAC	TGT	CCC	AGT		390
Gln	His	Met	Val	Thr	Asn	Ala	Ser	Ala	Val	Pro	Ser	Asp	Cys	Pro	Ser		
	105					110					115						
GAA	GAC	AAA	GAC	CTC	CTG	AAT	GAA	AAC	GTG	CAA	GTT	GGT	CTG	TTG	TTT		438
Glu	Asp	Lys	Asp	Leu	Leu	Asn	Glu	Asn	Val	Gln	Val	Gly	Leu	Leu	Phe		
	120				125					130					135		
GCC	TCG	AAA	GCC	ACC	GTC	CAG	CTC	ATC	ACC	AAC	CCT	TTC	ATA	GGA	CTA		486
Ala	Ser	Lys	Ala	Thr	Val	Gln	Leu	Ile	Thr	Asn	Pro	Phe	Ile	Gly	Leu		
				140					145					150			
CTG	ACC	AAC	AGA	ATT	GGC	TAT	CCA	ATT	CCC	ATA	TTT	GCG	GGA	TTC	TGC		534
Leu	Thr	Asn	Arg	Ile	Gly	Tyr	Pro	Ile	Pro	Ile	Phe	Ala	Gly	Phe	Cys		
			155					160					165				
ATC	ATG	TTT	GTC	TCA	ACA	ATT	ATG	TTT	GCC	TTC	TCC	AGC	AGC	TAT	GCC		582
Ile	Met	Phe	Val	Ser	Thr	Ile	Met	Phe	Ala	Phe	Ser	Ser	Ser	Tyr	Ala		
		170					175					180					
TTC	CTG	CTG	ATT	GCC	AGG	TCG	CTG	CAG	GGC	ATC	GGC	TCG	TCC	TGC	TCC		630
Phe	Leu	Leu	Ile	Ala	Arg	Ser	Leu	Gln	Gly	Ile	Gly	Ser	Ser	Cys	Ser		
	185					190					195						
TCT	GTG	GCT	GGG	ATG	GGC	ATG	CTT	GCC	AGT	GTC	TAC	ACA	GAT	GAT	GAA		678
Ser	Val	Ala	Gly	Met	Gly	Met	Leu	Ala	Ser	Val	Tyr	Thr	Asp	Asp	Glu		
	200				205					210					215		
GAG	AGA	GGC	AAC	GTC	ATG	GGA	ATC	GCC	TTG	GGA	GGC	CTG	GCC	ATG	GGG		726
Glu	Arg	Gly	Asn	Val	Met	Gly	Ile	Ala	Leu	Gly	Gly	Leu	Ala	Met	Gly		
				220					225					230			
GTC	TTA	GTG	GGC	CCC	CCC	TTC	GGG	AGT	GTG	CTC	TAT	GAG	TTT	GTG	GGG		774
Val	Leu	Val	Gly	Pro	Pro	Phe	Gly	Ser	Val	Leu	Tyr	Glu	Phe	Val	Gly		
			235					240					245				
AAG	ACG	GCT	CCG	TTC	CTG	GTG	CTG	GCC	GCC	CTG	GTA	CTC	TTG	GAT	GGA		822
Lys	Thr	Ala	Pro	Phe	Leu	Val	Leu	Ala	Ala	Leu	Val	Leu	Leu	Asp	Gly		
		250					255						260				

-continued

GCT Ala 265	ATT Ile	CAG Gln	CTC Leu	TTT Phe	GTG Val 270	CTC Leu	CAG Gln	CCG Pro	TCC Ser	CGG Arg 275	GTG Val	CAG Gln	CCA Pro	GAG Glu	AGT Ser	870
CAG Gln 280	AAG Lys	GGG Gly	ACA Thr	CCC Pro	CTA Leu 285	ACC Thr	ACG Thr	CTG Leu	CTG Leu	AAG Lys 290	GAC Asp	CCG Pro	TAC Tyr	ATC Ile	CTC Leu 295	918
ATT Ile	GCT Ala	GCA Ala	GGC Gly	TCC Ser 300	ATC Ile	TGC Cys	TTT Phe	GCA Ala	AAC Asn 305	ATG Met	GGC Gly	ATC Ile	GCC Ala	ATG Met 310	CTG Leu	966
GAG Glu	CCA Pro	GCC Ala	CTG Leu 315	CCC Pro	ATC Ile	TGG Trp	ATG Met	ATG Met 320	GAG Glu	ACC Thr	ATG Met	TGT Cys	TCC Ser 325	CGA Arg	AAG Lys	1014
TGG Trp	CAG Gln	CTG Leu 330	GGC Gly	GTT Val	GCC Ala	TTC Phe	TTG Leu 335	CCA Pro	GCT Ala	AGT Ser	ATC Ile	TCT Ser 340	TAT Tyr	CTC Leu	ATT Ile	1062
GGA Gly 345	ACC Thr	AAT Asn	ATT Ile	TTT Phe	GGG Gly 350	ATA Ile	CTT Leu	GCA Ala	CAC His	AAA Lys 355	ATG Met	GGG Gly	AGG Arg	TGG Trp	CTT Leu	1110
TGT Cys 360	GCT Ala	CTT Leu	CTG Leu	GGA Gly 365	ATG Met	ATA Ile	ATT Ile	GTT Val	GGA Gly	GTC Val 370	AGC Ser	ATT Ile	TTA Leu	TGT Cys	ATT Ile 375	1158
CCA Pro	TTT Phe	GCA Ala	AAA Lys 380	AAC Asn	ATT Ile	TAT Tyr	GGA Gly	CTC Leu	ATA Ile 385	GCT Ala	CCG Pro	AAC Asn	TTT Phe	GGA Gly 390	GTT Val	1206
GGT Gly	TTT Phe	GCA Ala	ATT Ile 395	GGA Gly	ATG Met	GTG Val	GAT Asp	TCG Ser 400	TCA Ser	ATG Met	ATG Met	CCT Pro	ATC Ile 405	ATG Met	GGC Gly	1254
TAC Tyr	CTC Leu	GTA Val 410	GAC Asp	CTG Leu	CGG Arg	CAC His	GTG Val 415	TCC Ser	GTC Val	TAT Tyr	GGG Gly	AGT Ser 420	GTG Val	TAC Tyr	GCC Ala	1302
ATT Ile 425	GCG Ala	GAT Asp	GTG Val	GCA Ala	TTT Phe 430	TGT Cys	ATG Met	GGG Gly	TAT Tyr	GCT Ala	ATA Ile 435	GGT Gly	CCT Pro	TCT Ser	GCT Ala	1350
GGT Gly 440	GGT Gly	GCT Ala	ATT Ile	GCA Ala	AAG Lys 445	GCA Ala	ATT Ile	GGA Gly	TTT Phe	CCA Pro 450	TGG Trp	CTC Leu	ATG Met	ACA Thr	ATT Ile 455	1398
ATT Ile	GGG Gly	ATA Ile	ATT Ile	GAT Asp 460	ATT Ile	CTT Leu	TTT Phe	GCC Ala	CCT Pro 465	CTC Leu	TGC Cys	TTT Phe	TTT Phe	CTT Leu 470	CGA Arg	1446
AGT Ser	CCA Pro	CCT Pro	GCC Ala 475	AAA Lys	GAA Glu	GAA Glu	AAA Lys	ATG Met 480	GCT Ala	ATT Ile	CTC Leu	ATG Met 485	GAT Asp	CAC His	AAC Asn	1494
TGC Cys	CCT Pro	ATT Ile 490	AAA Lys	ACA Thr	AAA Lys	ATG Met	TAC Tyr	ACT Thr	CAG Gln	AAT Asn	AAT Asn	ATC Ile 500	CAG Gln	TCA Ser	TAT Tyr	1542
CCG Pro 505	ATA Ile	GGT Gly	GAA Glu	GAT Asp	GAA Glu 510	GAA Glu	TCT Ser	GAA Glu	AGT Ser	GAC Asp	TGAGATGAGA TCCTCAAAAA			1595		
TCATCAAAGT GTTTAATTGT ATAAAACAGT GTTTCCAGTG ACACAACCTCA TCCAGAACTG 1655																
TCTTAGTCAT ACCATCCATC CCTGGTGAAA GAGTAAAACC AAAGGTTATT ATTTCTTTTC 1715																
CATGGTTATG GTCGATTGCC AACAGCCTTA TAAAGAAAAA GAAGCTTTTC TAGGGGTTTG 1775																
TATAAATAGT GTTGAAACTT TATTTTATGT ATTTAATTTT ATTAAATATC ATACAATATA 1835																
TTTTGATGAA ATAGGTATTG TGTAATCTA TAAATATTTG AATCCAAACC AAATATAATT 1895																
TCC 1898																

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

-continued

(A) LENGTH: 514 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met	Ala	Leu	Ser	Glu	Leu	Ala	Leu	Val	Arg	Trp	Leu	Gln	Glu	Ser	Arg
1				5					10					15	
Arg	Ser	Arg	Lys	Leu	Ile	Leu	Phe	Ile	Val	Phe	Leu	Ala	Leu	Leu	Leu
			20					25					30		
Asp	Asn	Met	Leu	Leu	Thr	Val	Val	Val	Pro	Ile	Ile	Pro	Ser	Tyr	Leu
		35					40					45			
Tyr	Ser	Ile	Lys	His	Glu	Lys	Asn	Ala	Thr	Glu	Ile	Gln	Thr	Ala	Arg
	50					55					60				
Pro	Val	His	Thr	Ala	Ser	Ile	Ser	Asp	Ser	Phe	Gln	Ser	Ile	Phe	Ser
65					70					75					80
Tyr	Tyr	Asp	Asn	Ser	Thr	Met	Val	Thr	Gly	Asn	Ala	Thr	Arg	Asp	Leu
				85					90					95	
Thr	Leu	His	Gln	Thr	Ala	Thr	Gln	His	Met	Val	Thr	Asn	Ala	Ser	Ala
			100					105					110		
Val	Pro	Ser	Asp	Cys	Pro	Ser	Glu	Asp	Lys	Asp	Leu	Leu	Asn	Glu	Asn
		115					120					125			
Val	Gln	Val	Gly	Leu	Leu	Phe	Ala	Ser	Lys	Ala	Thr	Val	Gln	Leu	Ile
	130					135					140				
Thr	Asn	Pro	Phe	Ile	Gly	Leu	Leu	Thr	Asn	Arg	Ile	Gly	Tyr	Pro	Ile
145					150					155					160
Pro	Ile	Phe	Ala	Gly	Phe	Cys	Ile	Met	Phe	Val	Ser	Thr	Ile	Met	Phe
			165						170					175	
Ala	Phe	Ser	Ser	Ser	Tyr	Ala	Phe	Leu	Leu	Ile	Ala	Arg	Ser	Leu	Gln
			180					185					190		
Gly	Ile	Gly	Ser	Ser	Cys	Ser	Ser	Val	Ala	Gly	Met	Gly	Met	Leu	Ala
	195						200					205			
Ser	Val	Tyr	Thr	Asp	Asp	Glu	Glu	Arg	Gly	Asn	Val	Met	Gly	Ile	Ala
	210					215					220				
Leu	Gly	Gly	Leu	Ala	Met	Gly	Val	Leu	Val	Gly	Pro	Pro	Phe	Gly	Ser
225					230					235					240
Val	Leu	Tyr	Glu	Phe	Val	Gly	Lys	Thr	Ala	Pro	Phe	Leu	Val	Leu	Ala
			245						250					255	
Ala	Leu	Val	Leu	Leu	Asp	Gly	Ala	Ile	Gln	Leu	Phe	Val	Leu	Gln	Pro
			260					265					270		
Ser	Arg	Val	Gln	Pro	Glu	Ser	Gln	Lys	Gly	Thr	Pro	Leu	Thr	Thr	Leu
		275					280					285			
Leu	Lys	Asp	Pro	Tyr	Ile	Leu	Ile	Ala	Ala	Gly	Ser	Ile	Cys	Phe	Ala
	290					295					300				
Asn	Met	Gly	Ile	Ala	Met	Leu	Glu	Pro	Ala	Leu	Pro	Ile	Trp	Met	Met
305					310					315					320
Glu	Thr	Met	Cys	Ser	Arg	Lys	Trp	Gln	Leu	Gly	Val	Ala	Phe	Leu	Pro
			325						330					335	
Ala	Ser	Ile	Ser	Tyr	Leu	Ile	Gly	Thr	Asn	Ile	Phe	Gly	Ile	Leu	Ala
			340				345						350		
His	Lys	Met	Gly	Arg	Trp	Leu	Cys	Ala	Leu	Leu	Gly	Met	Ile	Ile	Val
	355						360					365			
Gly	Val	Ser	Ile	Leu	Cys	Ile	Pro	Phe	Ala	Lys	Asn	Ile	Tyr	Gly	Leu
	370					375					380				

-continued

Ile	Ala	Pro	Asn	Phe	Gly	Val	Gly	Phe	Ala	Ile	Gly	Met	Val	Asp	Ser
385					390					395					400
Ser	Met	Met	Pro	Ile	Met	Gly	Tyr	Leu	Val	Asp	Leu	Arg	His	Val	Ser
				405					410					415	
Val	Tyr	Gly	Ser	Val	Tyr	Ala	Ile	Ala	Asp	Val	Ala	Phe	Cys	Met	Gly
			420					425					430		
Tyr	Ala	Ile	Gly	Pro	Ser	Ala	Gly	Gly	Ala	Ile	Ala	Lys	Ala	Ile	Gly
		435					440					445			
Phe	Pro	Trp	Leu	Met	Thr	Ile	Ile	Gly	Ile	Ile	Asp	Ile	Leu	Phe	Ala
	450					455					460				
Pro	Leu	Cys	Phe	Phe	Leu	Arg	Ser	Pro	Pro	Ala	Lys	Glu	Glu	Lys	Met
465					470					475					480
Ala	Ile	Leu	Met	Asp	His	Asn	Cys	Pro	Ile	Lys	Thr	Lys	Met	Tyr	Thr
				485					490					495	
Gln	Asn	Asn	Ile	Gln	Ser	Tyr	Pro	Ile	Gly	Glu	Asp	Glu	Glu	Ser	Glu
			500					505					510		
Ser	Asp														

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

- (v i) ORIGINAL SOURCE:
 - (A) ORGANISM: Synthetic primer

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTGACTAAAG TAGTCTGCC

19

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 17 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

- (v i) ORIGINAL SOURCE:
 - (A) ORGANISM: Synthetic primer

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TACAGAAATC CAGACGG

17

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 17 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

-continued

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v i) ORIGINAL SOURCE:

(A) ORGANISM: Synthetic primer

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CGTCTGGATT TCTGTAG

17

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: DNA (genomic)

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v i) ORIGINAL SOURCE:

(A) ORGANISM: Synthetic primer

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGCATGGTGC TTTCTAG

17

I claim:

1. An isolated DNA molecule encoding the amino acid sequence shown in FIG. 1 (SEQ ID NO: 2).

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,688,936

DATED : November 18, 1997

INVENTOR(S) : Edwards

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 8, line 60 "10g25" should read -10q25-

Column 24, line 8 "FV" should read -Fv-

Column 28, line 42 "3500xfor" should read -3500 x g for-

Column 33, line 21 "18 hours Fig." should read -18 hours. Fig.-

Column 41, line 58 "0.5M M" should read -0.5M-

Column 41, line 63 "2xstandard" should read -2 X standard-

Column 1, line 18 "BNS 90-11993" should read -BNS 90-11883-

Signed and Sealed this
Fifteenth Day of June, 1999

Attest:



Q. TODD DICKINSON

Attesting Officer

Acting Commissioner of Patents and Trademarks